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The Biochemistry *of* Malignant Tumors

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1943

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Brooklyn, N. Y.

PREFACE

THE present work has been written with full recognition of the great obstacles which lie in the path of anyone who attempts the integration of biochemical cancer research, more particularly so when the attempt is made to satisfy the expert in the field, as well as the reader who is only generally interested in biology or medicine, and who desires to obtain detailed information on the biochemical aspects of the problem of cancer.

The attempt to solve the problem of neoplasia long ago had extended beyond the boundaries of morphological pathology and clinical medicine. Biochemistry and experimental biology have been called upon to assist in this investigation of a disease which affects the lives of literally millions of suffering persons. To unite the somewhat confusing details of the many aspects of the cancer problem is exceedingly difficult.

Without indulging in undue optimism, it may be stated that the work of the last twenty years has brought about a remarkable increase of our knowledge of cancer. While no one discovery, thus far reported, can lay serious claim to having solved the cancer problem, the indefatigable efforts of scores of ingenious research workers have at least clarified some points, which indicate additional desirable research work which should be undertaken.

The most impressive results are the following: One, the discovery and study of carcinogens, i.e., chemical substances or forms of physical energy which induce neoplasia in a reproducible manner; two, investigations into the chemistry of neoplastic growth, emphasis being placed upon the possible detection of differences from nonmalignant growth processes, and efforts being made to establish connections between the anomalous growth pattern of tumors and the deviations from the normal which were believed to characterize metabolism and enzymology in neoplasia; three, analyses of the behavior of tumor hosts as to their resistance or predisposition to the development and growth of tumors, a field of research encompassing studies of hereditary factors, of immunological phenomena, and of systemic changes which were variously interpreted as either causing, accompanying or resulting from malignancy. The work comprised in these three categories appears to have yielded an affirmative answer to the question as to whether the biochemical approach is capable of definitely contributing to the exploration of the problems of neoplasia. Nevertheless, the question as to whether these trends of work, which occupy the main interest of present-day cancer research, will actually effect the solution of the tumor riddle is still open. Possibly, at some future time, these questions will be answered. It was thought appropriate to include less obvious aspects of chemical cancer research since it is conceivable that continued work may reveal them to be of much greater importance than is presently assigned to them.

As implied by the foregoing statement, the tendency has been to cover in this treatise the relationship of cancer to chemistry in the broadest meaning of both words, based on a collection of the literature as complete as possible. This was the intention—but it is by no means claimed that this aim has been actually reached. Shortcomings of the book, which may become apparent in this or other respects, are perhaps entitled to some forebearance, in view of the fact that this is the first attempt to present a work of this type to English-speaking countries, and because, generally speaking, books dealing with these topics have not been made available for a long time. The authors of this book have one previous work on the subject, the monograph "Die Wege und Ergebnisse chemischer Krebsforschung" (Vienna: Aeskulap-Verlag, 1936). The kind reception which this publication found in this and other countries encouraged the resumption of a similar undertaking at this time. Additional incentives were furnished by the undeniable progress of chemical cancer research during the last five years; finally it was thought that future work on these problems may profit to some extent from a presentation which summarizes what is known today, and which points out what is not known in this field.

A brief explanation may be given regarding the arrangement of the material chosen for this book. The three basic problems with which chemical cancer research must be concerned, and which command the main interest of clinician, biologist and chemist alike, are etiology, diagnosis and treatment of neoplastic diseases. Another point of view, possessing undeniable merit, might be based on biological principles, proceeding from the aspects of tumor development to those of tumor growth and to the response of the tumor host. In view of the enormous scope of the topics to be dealt with, it was thought preferable to employ a somewhat arbitrary grouping of the material in accordance with the general principles of biochemistry. At the same time it was felt necessary to include some data which at present do not appear to be directly related to chemistry—an overstepping of boundaries which seemed justified in the interest of a coherent presentation of allied fields of science.

Main emphasis has been placed on the work of the last twenty-five years, as being warranted by the achievements of general biochemistry. The literature has been systematically covered until the end of 1941, and a number of publications which appeared in the first half of 1942 are also included. At the same time, reference is made to work of older date, because of its historical interest, and because ideas expressed in these works have, in one form or the other, been the motive for investigations of more recent date.

Details of methodological nature have as a rule not been presented, but in this connection reference is made to the original articles or, whenever possible, to monographs containing the information.

A few words appear appropriate with regard to the manner chosen for quoting the authors of the studies surveyed. For the sake of avoiding the cumbersome accumulation of numerous proper nouns, the tendency was to eliminate the authors' names from the text as much as possible; instead, these names will be found by continuous numbers citing the references at the bottom of the pages. Unavoidable exceptions are represented by those instances in which scientific usage inseparably connects certain accomplishments with the name of their author, or by instances where the work in question forms a specialty original with the author, such as in cases of therapeutic preparations or of diagnostic procedures introduced by individual investigators. In any case, the mention, or the omission of mention, of an author's name should definitely not be interpreted as expressing a positive or negative evaluation of his work.

A special explanation is required regarding the circumstances of the collaboration with the co-author of this book—Dr. Robert Willheim. Dr. Willheim, formerly Associate Professor of Biochemistry at the University of Vienna, Austria, in 1939 accepted an appointment to the University of the Philippines in Manila. As previously mentioned, a book dealing with similar subjects was previously published under the joint authorship of Drs. Willheim and Stern. Subsequently, during a visit of Dr. Willheim to New York in 1939, plans were made for the publication of this book by revising, modifying, and bringing up to date the material contained in the older monograph. This intention assumed concrete form early in 1941, and based on earlier personal discussions and on continued exchange of ideas by correspondence, definite plans were drawn up for a collaboration of the two authors in this undertaking. Regrettably, however, extraneous circumstances caused a delay in the execution of these plans until, finally, the fateful events of December 1941 severed all possibilities of collaboration. Hence the actual drafting of this treatise remained for the other author (Dr. Kurt Stern). Nevertheless, the present writer feels wholly justified in having the resulting book appear under the joint authorship, as originally intended, a decision which was arrived at in view of the following facts: The contents of this book are in part based on those of the older book which was a product of close collaboration of the two authors; most of the views expressed here were formed during a period of more than ten years of joint experimental work in cancer research; finally, a thorough discussion of the most important aspects of the book had already taken place between the authors, who agreed as to the material to be included in the present volume. Nevertheless, in spite of these circumstances, the greater share of responsibility for the final result must be borne by the New York author who, at this time, wishes to express his hope that, if and when this book reaches his co-author, it may not appear altogether displeasing to him, and that a resumption of the original collaboration may become feasible at a not too distant. and in a happier future.

It must be stressed that this treatise is not intended to be a textbook, nor did the authors attempt to base the book as a whole on a "cancer theory" of their own. Naturally the special interests of the writers will become apparent in the discussion of those phases of cancer research which have formed the subject of their own experimental work. This refers principally to studies of certain aspects of tumor metabolism, of serodiagnostics of cancer, and of the part played by the reticulo-endothelial system in malignancy. Nevertheless, the tendency not to overemphasize these topics, and not to withhold the mention of conflicting evidence or statements has been adhered to. In general, the unpleasant task of discussing numerous instances, in which the experimental results as well as the theoretical interpretations of different research workers proved to be completely contradictory, too often presented itself. Efforts were made either to reconcile these discrepancies, or to find factors which may be responsible for them. Thus an endeavor was made, not only to enumerate the facts, but also to introduce a critical point of view. It is, however, openly admitted that the extent of such critical evaluations had to be curtailed—on the one hand, because of the vast scope of the subject to be surveyed, on the other hand, because clearest insight into some aspects of experimental work on special questions can be gained only by first-hand experience in these fields. Hence it may be concluded that the ideal form of a presentation of this kind would be a symposium resulting from the collaboration of a number of experts who are engaged in, and have essentially contributed to the elaboration of the various specialized branches of biochemical cancer research. However, circumstances beyond the control of the individual prevented the realization of such a plan. Therefore, the publication of the book in this form was undertaken in the sincere hope that the energy expanded upon it will, in spite of some limitations, be found justified by the benefits which may be derived: The detailed survey on the accomplishments and aims of biochemical cancer research may be expected both to facilitate the work of those engaged in these problems, and to arouse or to increase the interest of the biologist, the chemist and the clinician in this task of vital importance.

The author wishes to express his sincere thanks to Mr. H. Bennett, President of the Chemical Publishing Company, whose active interest rendered possible this publication. Best thanks are also tendered to Mr. T. E. R. Singer for editing the manuscript and to my wife for correcting the proofs.

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CHAPTER I

INORGANIC CHEMISTRY

THERE is a peculiar divergence of opinion regarding the evaluation of inorganic chemical findings in biology. The investigator coming from the exact natural sciences—physics, chemistry—expects that the results obtained in this field will satisfy, to a high degree, his urge for causality. Those, however, who are rooted in purely biological spheres—physiology, experimental pathology, clinical work—usually show a sceptical attitude towards the inorganic achievements of biochemistry. Both sides are able to support their views with adequate reasons: the chemist may point to the significant rôle of the electrolytes in colloid chemistry and in metabolic and enzymatic processes, whereas the biologist may refer to the deplorable confusion resulting from contradictory interpretations of numerous inorganic tissue analyses. In tumor chemistry also, both opinions are deserving of some consideration since, with the exception of a few definitely established facts which have a direct bearing on the tumor problem, there is a mass of findings the value of which is not in proportion to the work done.

The arrangement of the present material is based not only on chemical custom but also on viewpoints of tumor pathology with respect to possible carcinogenic or chemotherapeutic qualities of the elements treated. Generally, the material referring to the specific occurrence of the elements in the tumor and in the tumor organism is first reported, and then the possible significance of these substances is discussed. Finally, the experimental material is considered, both from the cancer producing and cancer curative aspects.

A. METALS

1. *Alkaline Metals and Alkaline Earths.*

Sodium. From an analytical point of view, most papers point to an increase of this element in tumors. An older statement, mentioning a relatively high sodium concentration in the ash of human cancers,[1] was corroborated by means of spectrometric methods.[2] An increased sodium content was noted also in Flexner-Jobling carcinomas of rats[3] and in other rat tumors.[4] The sodium concentration of the Rous sarcoma was found to be three times as high as the concentration in the surrounding muscles (300 mg. per cent, as compared with 100 mg. per cent).[5] This phenomenon has been connected to the increased hydration of the tumor tissue. No deviation from the normal sodium values was observed in whole blood and in other organs of fowl bearing Rous sarcoma,[5, 6] except in the serum in which the sodium level increased progressively until death. An increased sodium content was also found in the blood of tumor bearing rats.[4] A decreased sodium content was reported for the spleen of these animals.[7] However, a theory which, based on these findings, assumed the secretion by the spleen of a growth factor containing sodium, must be viewed with great reserve in view of lacking experimental evidence. No change of the sodium content in the plasma of tumor bearing mice was observed.[8] A slight decrease, however, was reported for rabbits affected with Brown-Pearce carcinoma.[9]

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[1] A. Robin: Compt. rend. Acad. d. sc. *156*: 334 (1913).

[2] D. C. A. Butts, T. E. Huff & F. Palmer, Jr.: Science *65*: 304 (1927).

[3] Y. Kimura: J. Biochem. *8*: 469 (1928).

[4] D. C. A. Butts, T. E. Huff & E. C. Manz: Bol. inst. de med. exper. para el estud. y trat. d. cancer *7*: 694 (1932).

[5] V. Morávek: Ztschr. f. Krebsforsch. *35*: 492; 509 (1932).

[6] H. M. Dyer & J. H. Roe: Am. J. Cancer *18*: 888 (1933).

[7] D. C. A. Butts: Libro de Oro, Buenos Aires, 1935, p. 179.

Only few and conflicting reports are available as to the sodium content of the serum in human malignancy; an alleged sodium decrease (98–130 mg. per 100 cc. as compared with normal values of 390 mg.)[10] was not confirmed by other investigators.[11, 12] An interesting finding was obtained for bone marrow; an augmented sodium content of the metastases-free bone marrow was observed in cancer patients.[13] Similar and more constant increases of the potassium in bone marrow are discussed later.

An influence of the sodium-ion on tumor growth has not yet been definitely established. Whereas an inhibiting effect was ascribed to sodium chloride application on rabbit sarcomas [14] and on transplantable mouse tumors,[15] the takes of intravenously injected Brown-Pearce carcinoma of the rabbit were increased by sodium chloride injections.[16] Certainly, these different arrangements of the experiments are not wholly comparable to each other, and consideration must also be given to the rôle of the chloride anion. Sodium administration has been said to increase the radiosensitivity of experimental tumors.[17]

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8. E. E. Jones & M. D. Rourke: J. Cancer Research *11*: 232 (1927).
9. F. Lasch & B. Lustig: Ztschr. f. Krebsforsch. *43*: 146 (1936).
10. N. Louros & Gaessler: Ztschr. f. Krebsforsch. *28*: 191 (1929).
11. R. C. Theis & S. R. Benedict: J. Cancer Research *8*: 499 (1924).
12. H. C. Pitts & H. R. Johnson: New England J. Med. *202*: 415 (1930).
13. T. Hoffmann: Biochem. Ztschr. *243*: 145 (1931).
14. R. Jinguu: Tr. Jap. Path. Soc. *21*: 743 (1931).
15. H. Binhold: Ztschr. f. Krebsforsch. *38*: 587 (1933).
16. W. A. Collier & A. Cohn: Ztschr f. Krebsforsch. *38*: 291 (1933).
17 F. Eichholtz: Biochem. Ztschr. *235*: 170 (1931); Messende Methoden in der Carcinomforschung, Niemeyer, Halle a. S., 1933.

Potassium and calcium in tumor tissues. Investigations on the potassium content of malignant neoplasms are, as a rule, closely connected to those regarding the calcium content, since an antagonism of these two cations has been considered an established fact in other fields of physiological chemistry (F. K. Kraus, S. G. Zondek). This antagonism is expressed by the potassium calcium ratio which assumed special significance for tumor chemistry. A number of authors,[18-21] in this connection, showed that in malignant tumors a potassium increase parallels a calcium decrease. The possibility was even suggested of establishing a direct proportion between the tumor malignancy and the increase of the potassium calcium ratio.[19] A potassium calcium proportion of 2:1 or 3:2 was considered to be optimal for rapid growth. Correspondingly, rapidly growing tumors were claimed to show an especially low calcium concentration [21] and this was confirmed by ash analyses.[22] The rise of the potassium calcium ratio during the period of accelerated growth was principally attributed to a potassium increase.[23] A marked calcium decrease was also noted in Rous fowl tumors, as compared with the healthy, tumor-free muscle.[24] In the case of Rous tumors and transplantable mouse carcinomas, calcium feeding resulted in a definite calcium increase in the tumors. No relation was found to exist between the age and the calcium content of mouse tumors. Calcium decrease and potassium increase were also established for a rabbit sarcoma [25] while a

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18. S. P. Beebe: Am. J. Physiol. *12*: 167 (1904/05).
19. G. H. A. Clowes & W. S. Frisbie: Am. J. Physiol. *14*: 173 (1905).
20. M. Tracy: J. Med. Research *14*: 447 (1905/06).
21. N. Waterman: Arch néerland. physiol. *5*: 305 (1921).
22. A. Policard & D. Pillet: Compt. rend. Soc. de biol. *92*: 273 (1925).
23. A. H. Roffo & J. Lasserre: Bol. inst. de med. exper. para el estud. y. trat. d. cáncer *2*: 221 (1926).
24. A. Goldfeder: Ztschr. f. Krebsforsch. *27*: 503 (1928).
25. T. Nagaoka, M. Hirata & S. Fukunaga: Tr. Jap. Path. Soc. *19*: 657 (1929).

conflicting statement of calcium increase and unchanged potassium content was made for the Flexner-Jobling rat carcinoma.[8] However, potassium determinations performed in the dried material of normal organs and of various malignant neoplasms revealed a very marked difference; while normal tissues yield a potassium concentration of 1.4%, malignant tumors showed an average of 2.3%, i.e., an increase of more than 50%.[26] Comparing the potassium content of various tumors, it was stated that the concentrations obtained are directly proportional to the malignancy of the tumor and inversely proportional to the stroma content; therefore they are markedly higher in cancers derived from glandular structures and from mucous membranes than in skin cancer.[27] A potassium increase in carcinomas and sarcomas was also observed by histochemical methods, while the calcium content showed less characteristic variations.[28] Transplantable hepatomas of rats, produced by o-aminoazotoluene, were found to be decidedly richer in potassium than normal liver tissue, while the calcium content did not undergo changes.[29]

This disturbance of the potassium calcium ratio in malignant tumors, established by numerous investigations, is by no means an insignificant phenomenon. Experiments on parthenogenesis [30, 31] have shown the importance of these cations for development and growth. The growth promoting effect of the potassium ion in plants and lower animals has been known for a long time.[32] The potassium calcium ratio

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26. L. Kovács & M. Ambrus: Magyar orvosi arch. *32*: 220 (1931); abstr. Rona's Ber. *62*: 290 (1931).
27. A. Epstein: Ztschr. f. Krebsforsch. *38*: 63 (1932).
28. W. Schulze & H. Zschau: Frankfurt. Ztschr. f. Path. *48*: 51 (1935).
29. T. Fujiwara, W. Nakahara & S. Kishi: Gann *31*: 51 (1937).
30. J. Loeb: Biochem. Ztschr. *5*: 351 (1907).
31. R. S. Lillie: Am. J. Physiol. *26*: 106 (1910); cf. R. Hoeber: Physikalische Chemie der Zelle und Gewebe, ed. 5, 1924, pp. 624.
32. B. Moore, H. E. Roaf & R. E. Knowles: Biochem. J. *3*: 279 (1908).

also increases in normal organs of higher animals (pigeons) in proportion to the growth rate, whereas it decreases with aging. Generally speaking, the potassium content parallels the functional activity of an organ.[33] In satisfactory agreement with these facts, regenerative tissues also showed an increased potassium calcium ratio.[34]

A statement contradictory to earlier findings [24] must be recorded for the Rous tumor. Comparative potassium and calcium analyses of this tumor and of the surrounding healthy muscle showed a decrease to ⅙ of the normal value of the potassium concentration in the tumor, and a calcium increase of more than three times the content in normal muscle.[35] In view of the different functions of potassium and calcium in muscle and in tumor, the justification of a comparison of these tissues was questioned.[36]

Potassium and calcium in the organism of the tumor host. In accordance with the widely recognized tenet of considering neoplasia a systemic and not a purely local disease, many studies have been devoted to the inorganic constitution of the organism of the tumor host. By means of organ analyses of tumor-bearing mice results were obtained supporting the assumption of a general chemical change in the body. The potassium calcium ratio, which varies in organs of normal mice between 5 (liver) and 0.05 (connective tissue), was found almost constantly increased in tumor hosts and even reached values of 57.[37] The reports on the calcium level in the serum of cancer patients, however, are much more equivocal and far more subject to interpretation. It must be realized that in these clinical investigations the analyzed cases represented widely varying tumor localizations (skeleton involvement) and different stages of the disease, and are therefore not easily comparable. Correspondingly, some authors [38–41, 10, 12] reported unchanged serum calcium concentrations, while other publications indicate a lowered serum calcium level in cancer patients.[42–50, 16] The latter findings are most apparent in extreme cachexia.[51–53] Similarly it was observed that a hypocalcemia (below 9 mg. per cent) found in 15 out of 31 analyzed cancer patients showed a distinct relation to the loss of body weight, i.e., to nutritional disturbances.[54] The hypocalcemia has been claimed to be more frequently present in patients of over 40 years of age.[55] A hypercalcemia observed in the presence of osteolytic bone metastases [56] is easily understood. The low calcium values found

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[33] L. Kaufman & M. Laskowski: Biochem. Ztschr. *242*: 424 (1931).

[34] F. M. Brikker & Y. A. Lazaris: Ztschr. f. Krebsforsch. *34*: 35 (1931).

[35] V. Morávek: Ztschr. F. Krebsforsch. *35*: 386; 626 (1932).

[36] A. Lasnitzki: Die Bedeutung der Kationen für den Stoffwechsel, 1st Internat. Cancer Congress, Madrid, 1933.

[37] M. Wolf: Compt. rend. Acad. d. sc. *176*: 1932 (1923).

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[38] O. Krehbiel: J. Cancer Research *5*: 199 (1920).

[39] N. Waterman: Biochem. Ztschr. *133*: 535 (1922).

[40] A. Schepetinsky & M. Kafitin: Arch. F. Gynäk. *136*: 379 (1929).

[41] G. Sannicandro: Ann. di ostet. e ginec. *54*: 1603 (1932).

[42] G. L. Rohdenburg & O. Krehbiel: J. Cancer Research *6*: 223 (1921).

[43] H. Leicher: München. med. Wchnschr. *72*: 762 (1923).

[44] L. Blum & A. Klotz: Compt. rend. Soc. de Biol. *89*: 1335 (1923).

[45] R. C. Theis & S. R. Benedict: J. Biol. Chem. *61*: 63 (1924).

[46] R. Paolucci: Riforma med. *41*: 937 (1925).

[47] K. Svehla: Compt. rend. Soc. de biol. *94*: 980 (1926).

[48] L. Gunther & D. M. Greenberg: Arch. Int. Med. *46*: 67 (1930).

[49] H. Guthmann, H. Winkler & N. Grzimek: Arch. f. Gynäk. *155*: 185 (1933).

[50] S. I. Aisenberg, D. G. Krizschin, D. O. Gitis & Z. M. Erusalimski: Med. Z. vseukrain. Akad. nauk *4*: 1147 (1935): abstr. Ztschr. f. Krebsforsch. *44*: Ref. 68 (1936).

[51] I. Nikolskij: Vopr. Onkol. *2*: 215 (1929); abstr. Ztschr. f. Krebsforsch. *33*: Ref. 26 (1931).

[52] M. Tabanelli: Riv. d. pat. sper. *10*: 234 (1933); abstr. Am. J. Cancer *24*: 152 (1935).

[53] S. Tomisawa: Mitt. a. d. med. Akad. zu Kioto *18*: 94 (1936).

[54] H. Jackson, Jr. & F. H. L. Taylor: Am. J. Cancer *19*: 378 (1933).

[55] C. de Fermo: Minerva med. *1*: 864 (1930).

[56] A. B. Gutman, T. L. Tyson & E. B. Gutman: Arch. Int. Med. *57*: 414 (1936).

in the sera of patients with gastric cancer were correlated to the gastric anacidity occurring in this condition.[57] Incipient cases of uterine cancer exhibited normal serum calcium, but with advancing disease a decrease was noted which was considered a secondary phenomenon depending on disturbances of the protein metabolism (mainly on the hypoproteinemia present in these cases [58, 59]). From all these results it may clearly be seen that changes of the serum calcium level in tumor patients, if present, are not specific, but are of secondary nature and, therefore, no etiologic or diagnostic conclusions can be drawn from them.

This view appears also to be supported by the results of studies performed with experimental animal tumors. Although in sera of rabbits painted with tar a diminished serum calcium concentration was noted from the start of the cancerization,[60] and a similar behavior was described in rabbits inoculated with Brown-Pearce carcinoma,[61] other investigators arrived at different conclusions. No change of the serum calcium level was found in tumor-bearing rabbits,[62] or else only a slight decrease parallel to the tumor advancement (rabbit Kato sarcoma [65]).

The ionic state of calcium is apparently not materially affected in serum of cancer patients as it proved to be approximately 62 per cent dialysable.[36, 48] Reding,[67] however, calcu-

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57. W. Thiele: Klin. Wchnschr. *16*: 165 (1937).
58. R. O. Bowman & H. C. Pitts: Am. J. Obst. & Gyn. *32*: 957 (1936).
59. Z. Mizuno: Jap. J. Obst. & Gyn. *21*: 244 (1938).
60. A. Rémond, M. Sendrail & Lassalle: Compt. rend. Soc. de biol. *93*: 1061 (1925).
61. A. R. Harnes: J. Exper. Med. *50*: 109 (1929).
62. K. Suzuki: Tr. Jap. Path. Soc. *22*: 985 (1932).
63. Z. Mizuno: Jap. J. Obst. & Gyn. *21*: 250 (1938).
64. Z. Mizuno: Jap. J. Obst. & Gyn. *21*: 328 (1938).
65. Z. Mizuno: Jap. J. Obst. & Gyn. *21*: 332 (1938).
66. A. H. Roffo & L. Correa: Bull. Soc. chim. biol. *7*: 522 (1925).
67. R. Reding: Le terrain cancereux and cancérisable, Masson & Cie, Paris, 1932.

lating the amount of ionized calcium according to the Rona and Takahashi equation ($Ca^{++} = k \times \frac{H^{+}}{HCO3^{-}}$), found an average value of 1.77 mg. in cancer cases, as contrasted with 2.28 mg. per 100 c.c. in normal persons.

Determinations of the potassium content of the serum of cancer patients yielded deviations from the norm in only a minority of the cases and in these instances also no correlation to the tumor process could be established.[68, 12, 54] However, an increased potassium concentration in red blood cells was found in 40 per cent of cancer patients.[68, 69] Other authors [11, 40, 49] reported on a slight decrease of the potassium level in approximately 40 per cent of the analyzed cancer sera. In complete contradiction to these statements, increased potassium concentrations in the serum of tumor patients were reported, and values of 32—59 mg. per cent [70] and of 22—25 mg. per cent [50] were given. It is possible that these extremely high values were due to errors in method. At any rate, the conflicting claims mentioned make it desirable to renew these studies, although it does not appear likely that much valuable information will be gained from them. No specific change of the potassium and calcium content has been detected in the serum of fowl affected with Rous sarcomas.[6]

An interesting observation was reported in relation to the potassium content of bone marrow. The potassium concentration of this tissue decreases to ¼th to ⅕th of the original value after termination of growth. Concomitant with tumor development, however, the potassium content of the bone marrow has been claimed to increase to the extent of

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68. A. Rémond & E. Cantegril: Compt. rend. Soc. de biol. *103*: 764; *104*: 293 (1930).
69. E. Cantegril: Progrès méd. *1*: 893 (1931).
70. C. Giacobbe: Riforma med. *1*: 751 (1931).

at least twice the amount of potassium present in the corresponding tissue of cancer-free persons.[71] On the basis of these findings, a theory of carcinogenesis was proposed, and a therapy also recommended, which will be discussed later in the section on the Reticulo-endothelial System.

Etiologic and therapeutic implications. Although the investigations enumerated on changes of the inorganic constitution connected with the tumor process have by no means led to conclusive results, attempts have been made to gain from them both insight into the etiology and therapy of cancer. Numerous purely speculative theories are concerned with the alleged changes of the tumor chemistry. Thus a potassium augmentation [72] and its radioactivity [73] were made responsible for carcinogenesis. In particular, the frequent association of gall bladder carcinoma with cholelithiasis was tentatively explained by the potassium content of the gall stones.[74] Experimental work, however, does not support these speculative theories, inasmuch as in the Jensen rat sarcoma the radioactive potassium-isotope was found in the same concentration as in normal muscle.[75] According to a recent report, the heavy isotope K^{41} has been found to be diminished in Jensen rat sarcomas, in mouse sarcomas 37, and in the muscles of the tumor-bearing animals, when compared with mineral potassium or with muscles of animals free of tumors.[76] Analogous results were derived from comparative analyses of human neoplasms and normal human tissues. The ratio K^{39}/K^{41} was higher and the values for the heavy potassium isotope were found to be lower in the tumors than in mineral potassium or in normal tissues.[76a] However, no definite opinion can be offered, as yet, as to the significance of these findings.

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[71] P. Rosenstein & H. Köhler: Med. Klin. *27*: 1095 (1931).

[72] H. Zwaardemaker: Am. J. Physiol. *45*: 147 (1918); Klin. Wchnschr. *1*: 535; 594 (1922).

[73] Magrou, quot. G. Joannović: Klin. Wchnschr. *2*: 2301 (1923).

[74] S. Lazarus-Barlow: quot. A. H. Roffo & J. Thomas: La chimie du cancer, Vigot frères, Paris, 1933, p. 66.

[75] A. Lasnitzki: Am. J. Cancer *35*: 363 (1939).

[76] A. Lasnitzki & A. K. Brewer: Cancer Research *1*: 776 (1941).

On the other hand, a primary potassium deficiency of the cells was assumed to be a factor of disposition to cancer.[77] Finally, the increased potassium content of tumor tissue was correlated with its radiosensitivity, and an artificial potassium augmentation of tumors was proposed for the purpose of achieving a greater effect of radiation therapy.[78] Actually, the administration of potassium salts to tumor animals resulted in an increased radiosensitivity, but this effect could be achieved still more easily by means of calcium.[17] This agrees well with histochemical analyses showing a direct relation between the inorganic content of cells and radiosensitivity.[79] A rise of the calcium concentration was observed after X-ray treatment of cancer tissue,[80] which would prove that effective treatment results in a reversion of the chemical constitution to the norm. However, this finding was subject to contradiction.[81] The potassium content of irradiated tumors was also found to undergo changes; after an initial increase, considered as inflammatory reaction, a marked decrease was observed and, at the same time, the vitality of the tumor cells appeared diminished.[27] Therefore, it is understandable that calcium administration was recommended and tried in human malignancy,[82] especially as a

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76a. A. Lasnitzki & A. K. Brewer: Cancer Research *2*: 494 (1942).

77. J. L. Bulkley: Cancer (New York) *4*: 149 (1927).

78. P. Ottonello: Radiol. med. *17*: 580 (1930); abstr. Ztschr. f. Krebsforsch. *33*: Ref. 37 (1931).

79. I. A. B. Cathie: J. Path. & Bact. *48*: 1 (1939).

80. L. Schönholz & H. Hirsch: Strahlentherapie *34*: *273* (1929).

81. M. A. Magath & M. J. Kolomijetz: Ztschr. f. Krebsforsch. *30*: 457 (1930).

82. R. J. Behan: Schweiz. med. Wchnschr. *64*: 311 (1934); Am. J. Surg. *19*: 301 (1933).

supporting measure for radiation therapy.[83] According to one report, calcium application in a case with bone metastases caused a roentgenologically proved sclerosis. Relief of pain without the use of analgesics, alkaloids or X-ray therapy, and almost complete regeneration of the bone defects [84] were seen from this therapy.[85] Calcium precipitates were formed by means of intratumoral injections of soluble calcium salts and phosphates into breast cancers. In a few cases it is alleged that this treatment caused ulceration, scarification and cure of the tumors.[86] The scarcity of the material and the intratumoral application make necessary a sceptical interpretation of this claim.

A brief report [87] of three inoperable tumors, in which medication of liquor potassii produced reduction of the size of metastases and general improvement, can scarcely be considered a basis for the recommendation of a potassium therapy for cancer.

Effect of potassium and calcium on experimental tumors. The biological antagonism between potassium and calcium is very clearly expressed by the influence exerted by them on animal tumors. Tumor fragments treated with calcium salts preceding their inoculation showed inhibited growth.[88, 89] Calcium injections prior to tumor transplantation prevented tumor growth in mice and rats.[90, 91] The filtrable agent of Rous sarcoma was inactivated by some calcium compounds

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[83] W. Hug: Strahlentherapie *48*: 118 (1933).

[84] A. Brunschwig: Am. J. Cancer *25*: 721 (1935).

[85] E. E. Downs, H. Wammock & R. T. Artman: Am. J. Roentgenol. *37*: 790 (1937).

[86] R. Vincent & H. Chauhard: Bull. Acad. de méd., Paris *121*: 638 (1939).

[87] D. M. Gall: Lancet *206*: 1104 (1924).

[88] W. Cramer: Biochem. J. *12*: 210 (1918).

[89] K. G. Falk, H. M. Noyes & K. Sugiura: J. Biol. Chem. *53*: 75 (1923).

[90] Katase, Kawamura & Mizutani: Verh. d. jap. path. Ges. *10*: 107 (1920).

[91] T. S. Paik: Tr. Jap. Path. Soc. *20*: 653 (1930).

(CaO, Ca $(H_2PO_4)_2$).[92] In this case, however, a purely physical adsorption appears possible. On the other hand, pretreatment of mouse carcinoma with potassium salts enhanced takes and the growth of the tumor.[93–95] A potassium rich diet markedly increased the takes, accelerated tumor growth and promoted metastases formation.[96] The peroral or parenteral application of calcium lactate inhibited the growth of mouse tumors, whereas potassium citrate exerted the opposite influence.[97, 98] The production of liver cancer in rats by means of o-aminoazotoluene was delayed when calcium was added to the diet.[99] Following calcium injections (together with gelatin and trypan blue) hyalinization, calcification and growth inhibition of the Jensen rat sarcoma were observed.[100] Similarly, a growth inhibiting effect on a rabbit sarcoma was ascribed to calcium salts.[14] An extremely potassium deficient diet caused growth inhibition of inoculated mouse tumors,[101] and the opposite reaction occurred in mice kept on a very potassium rich diet where a more rapid take of grafts, potassium increase in the tumors, and augmented metastases formation were noted.[102] A potassium deficient diet strongly inhibited the growth of transplanted mouse tumors. Large, rapidly growing tumors, however, were not significantly influenced by removing potassium from the diet.[103] This finding leads to a very different picture which becomes apparent when experiments have dealt with animal tumors already developed. Here neither potassium nor calcium injections showed any effect on the tumor growth.[104] The inefficiency of calcium application was established in an experiment with more than 1200 tumor-bearing mice [105] and, similarly, calcium injections were ineffective in rat sarcomas.[106] Moreover, the prophylactic value of calcium treatment was not always confirmed.[105] The implantation success of Brown-Pearce rabbit carcinoma was not affected by calcium administration.[16] One experimenter even reported a growth promoting effect of calcium injections on mouse tumors.[15] The results of tissue culture studies, however, confirmed the growth inhibiting quality of calcium as expressed in most of the experiments mentioned. Normal, as well as neoplastic tissue cultures showed a growth promotion by potassium addition and an opposite influence of calcium.[107, 108] In a recent investigation, the following results were obtained when potassium was substituted in tissue cultures for sodium; under these conditions, normal embryonic mesenchyme and heart tissue of newborn mice grew more slowly than control cultures, whereas the growth of three different transplanted mouse carcinomas was stimulated by the potassium substitution; cultures of two mouse sarcoma strains occupied an intermediate position between the normal and the carcinomatous tissues.[108a]

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[92] M. R. Lewis & H. B. Andervont: Bull. Johns Hopkins Hosp. *42*: 191 (1928).

[93] J. Troisier & M. Wolf: Compt. rend. Soc. de biol. *86*: 651 (1922).

[94] L. Nègre: Compt. rend. Soc. de biol. *86*: 746 (1922).

[95] A. Lasnitzki: Ztschr. f. Krebsforsch. *27*: 115 (1928).

[96] M. Handel: Ztschr. f. Krebsforsch. *21*: 281 (1924).

[97] M. Goldzieher; Verh. d. deutsch. path. Ges. *15*: 223 (1912).

[98] M. Goldzieher & E. Rosenthal: Ztschr. f. Krebsforsch. *13*: 321 (1913).

[99] Z. Mizuno: Jap. J. Obst. & Gyn. *21*: 338 (1938).

[100] R. A. Hunter: J. Path. & Bact. *43*: 35 (1936).

[101] E. Langfeldt: Skandinav. Arch. f. Physiol. *55*: 275 (1928).

[102] A. Epstein: Ztschr. f. Krebsforsch. *38*: 535 (1933).

[103] A. A. Liebow, W. J. MacFarland & R. Tennant: Yale J. Biol. & Med. *13*: 523 (1941).

Correlation of potassium and calcium to tumor metabolism. Several papers have dealt with the problem of whether these two cations influence the metabolic processes of tumors. In this respect, an inhibition of tumor glycolysis by calcium[109] and an opposite effect by potassium were reported.[110] Extensive studies, however, showed that both potassium and calcium are capable of materially promoting the respiration and glycolysis of tumor, as well as of normal tissue.[111, 112, 86] This apparent synergism of both cations was explained by the assumption that potassium represents the really effective factor, whereas calcium exerts only an indirect action by tightening the cell membranes, thus preventing the diffusion of the active potassium from the cells.[113] Consideration of the fact that the sealing of the cell membranes by calcium would also impede potassium intake from the surrounding medium might perhaps offer an explanation for the aforementioned conflicting statements regarding the calcium influence on tumor growth. The effect of the two cations upon metabolism has been explained by still other workers,[114] on the basis of physico-chemical structural changes in the protoplasma, mainly as to the permeability.

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[104] G. L. Rohdenburg & O. Krehbiel: J. Cancer Research *7*: 417 (1923).

[105] M. J. Shear: Pub. Health Rep. *48*: 1103 (1933).

[106] G. Koerbler: Compt. rend. Soc. de biol. *92*: 628 (1925).

[107] A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat d. cáncer *1*: 537 (1925).

[108] S. Ricca: Arch. ital. med. sper. *2*: 815 (1938).

[108a] R. Tennant & A. A. Liebow: Yale J. Biol. & Med. *14*: 553 (1942).

Actually, the changes of the potassium and calcium content were assumed to be responsible for deviations of the physico-chemical tumor structure, especially as expressed by an augmented permeability, increased electric conductivity, and diminished electric polarizing capacity of malignant tissues.[115, 116] This view appears to be supported by experiments in which these abnormal physico-chemical properties of tumors could be caused to revert to the norm by means of calcium addition in vitro.[115]

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109. N. Waterman: Arch. néerland. physiol. *9*: 573 (1924).
110. M. Nakamura & K. Suzuki: Tr. Jap. Path. Soc. *20*: 677 (1930).
111. A. Lasnitzki & O. Rosenthal: Biochem. Ztschr. *207*: 120 (1929).
112. A. Lasnitzki & O. Rosenthal: Biochem. Ztschr. *262*: 203 (1933).
113. A. Lasnitzki: Brit. J. Exper. Path. *18*: 423 (1937); Am. J. Cancer *32*: 513 (1938).
114. F. Dickens & G. D. Greville: Biochem. J. *29*: 1468 (1935).
115. N. Waterman: Ztschr. f. Krebsforsch. *19*: 101 (1922).
116. A. H. Roffo & H. Degiorgi: Néoplasmes *4*: 202 (1925).

Several papers refer to correlations between mineral metabolism and endocrine glands. Quite some time ago, the inhibition of tumor growth, observed after parathyroid hormone application, was attributed to a calcium augmentation in the body.[97] A treatment of this kind was recommended for prophylaxis and therapy of cancer,[117] but it does not seem to be at all rational in view of experiments showing a growth promotion of Flexner-Jobling rat carcinomas after parathyroid hormone injections, in spite of a calcium increase in the tumors.[118] Similarly, the use of parathyroid hormone for clinical purposes in cancer was discouraged, as this hormone might bring about a calcium mobilization from the bones, and hence facilitate formation of bone metastases.[84] An interesting correlation of mineral content to endocrine glands was revealed by studies which indicated a lowered potassium concentration of tumors after castration of the animals.[119] This finding might be correlated to the beneficial effect on malignant tumors attributed by many authors to castration (cf. Chapter on Hormones). On the other hand, administration of estrogens or of testosterone propionate to patients suffering from bone metastases of breast cancer was seen to cause a definite rise of the serum calcium values and of the urinary calcium excretion. These effects were interpreted as being a consequence of a stimulated growth of the bone metastases involving an accelerated osteolysis and a flooding of the circulation with products of bone destruction.[119a]

An attempt was made to influence the calcium content of tumors also by means of vitamin D, a factor of decisive importance in inorganic metabolism. These experiments, however, failed completely to influence tumor growth, notwithstanding a noted moderate[120] or more marked [121, 122] calcium increase in the tumors. An interesting phenomenon, encouraging further analysis, was revealed by the report that injections of $CaCl_2$ or of vitamin D, respectively, caused a greater increase of the serum calcium in sarcoma-bearing rabbits than in normal ones.[123] Further studies must be awaited before deciding whether this depends on an impaired ability of calcium fixation of tumor animals, or on a disturbance of calcium excretion.

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[117] R. Reding & A. Slosse: Ann. soc. roy. sc. med. Bruxelles *9*: 231 (1929).

[118] A. Goerner & B. G. P. Shafiroff: J. Cancer Research *12*: 294 (1928).

[119] M. Loeper, R. Turpine & Zizine: Compt. rend. Soc. de biol. *93*: 94 (1925).

[119a] J. H. Harrow & H. Q. Woodard: J. A. M. A. *118*: 341 (1942).

Magnesium. The available reports do not reveal characteristic changes of the magnesium content of malignant neoplasms. While in older studies exact magnesium determinations in tumors were not feasible because of analytical difficulties,[18, 19] later, most authors [124, 17] found magnesium values within the normal range in various animal tumors.

The serum magnesium level of cancer patients was found either unchanged,[125] or only slightly lowered.[126, 127] As the latter finding was also obtained in pulmonary tuberculosis, and in tertiary lues,[126] this might be viewed as an expression of cachexia only. Similarly, no great significance can be attributed to a slight magnesium increase observed in the sera of 15 patients suffering from uterine carcinoma.[10] In a recent study, the average magnesium value in the sera of 23 patients with malignant tumors was determined as 2.10 mg. per cent, which is slightly below the normal average of 2.48 mg. per cent.[127a] A magnesium decrease was noticed in the Rous sarcoma whereas the serum of these animals contained normal magnesium amounts,[128] and the serum magnesium level of Brown-Pearce carcinoma rabbits was reported as markedly decreased.[9] In view of the therapeutic experiments discussed later, it may be noted here that injection or feeding of magnesium salts failed to increase the concentration of this element in rat tumors.[129–131]

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[120] A. Goerner: J. Cancer Research *14*: 545 (1930).

[121] F. M. Brikker & Y. A. Lazaris: Vopr. Onkol. *4*: 174 (1931); abstr. Ztschr. f. Krebsforsch. *35*: Ref. 93 (1932).

[122] F. M. Brikker & Y. A. Lazaris: Ztschr. f. Krebsforsch. *37*: 423 (1932).

[123] S. E. Rhee & C. H. Kim: Tr. Jap. Path. Soc. *22*: 974 (1932).

[124] A. Bolaffi: Tumori *16*: 420 (1930).

[125] A. Klotz: Compt. rend. Soc. de biol. *89*: 1337 (1923).

[126] E. Giovannoni: Rass. clin. *31*: 167 (1932); abstr. Ztschr. f. Krebsforsch. *39*: Ref. 20 (1933).

[127] J. G. Savitsky: Vestn. Rentgenol. *16*: 112 (1936); abstr. Ztschr. f. Krebsforsch. *45*: Ref. 152 (1937).

The material dealing with experimental and therapeutic attempts to influence the tumor process by magnesium is much more extensive than the analytical data mentioned. Delbet [132] ascribed a great importance to this element for carcinogenesis in his magnesium deficiency theory. Primarily, this view was claimed as being supported by chemical soil analyses based on the idea that a magnesium poverty of the soil, of the drinking water, and of the cereals should cause a magnesium deficiency in the body thus inducing a disposition to cancer. It was stated in this connection that in certain parts of France, Italy,[133] England,[134] and Germany [135] a reverse ratio existed between magnesium content of the soil and cancer mortality. Likewise, an attempt was made to explain an alleged rarity of malignant neoplasms in

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127a. V. G. Haury & A. Cantarow: J. Lab. & Clin. Med. *27*: 616 (1942).
128. V. Morávek: Ztschr. f. Krebsforsch. *36*: 529 (1932).
129. A. Bolaffi: Tumori *16*: 201 (1930).
130. K. Lang: Ztschr. f. klin. Med. *122*: 206 (1932).
131. F. Eichholtz & W. Kauderer: Biochem. Ztschr. *276*: 326 (1935).
132. P. Delbet: Pag. san. *1*: 24 (1928); Bull. Acad. de. méd., Paris *100*: 793 (1928).
133. L. Robinet: Bull. Assoc. franç. p. l'étude du cancer *18*: 243 (1930); *20*: 434 (1931).
134. L. Robinet: Bull. Acad. de méd., Paris *106*: 48 (1931).
135. L. Robinet: Bull. Assoc. franç. p. l'étude du cancer *21*: 464 (1932).

Egypt because of the high magnesium concentration of the soil of this particular country. The use of potassium-rich and magnesium-poor artificial fertilizer was held responsible for the high cancer incidence in modern countries.[136] But in contrast to these claims, extensive studies of the magnesium content of soil, water and food substances in Alsace did not confirm any connection between these analyses and cancer mortality.[137] Generally, the theory named is open to several objections; firstly, the statistical reliability appears doubtful; secondly, all other climatic and geographical factors influencing cancer incidence are neglected; and, finally, a special weakness of this theory may be seen in that food consumed at a certain location is only domestic to a small extent, while most of it is imported from distant regions.

The same lack of conclusive evidence in support of the alleged antiblastic quality of magnesium is apparent by the results from extensive animal experimentation. The positive results were reported almost exclusively by one group of authors, and were not confirmed by numerous re-examinations. The growth of transplanted mouse tumors was impeded by intra-muscular $MgSO_4$-injections,[138] tar cancerization of mice was inhibited by implantation of metallic magnesium and was prevented by $MgSO_4$-injections.[139] The most marked effects were reported by Delbet and his collaborators; magnesium chloride [140] and especially a mixture of some magnesium salts (Delbet's solution,[141]), parenterally or perorally administered, inhibited the growth and lowered the virulence of animal tumors. Likewise, the tar carcinoma of rabbits was impeded by magnesium application.[142, 143] Animals prophylactically treated with magnesium were claimed to exhibit an increased resistance against inoculated tumors[144] and in tissue cultures it was the neoplastic cells which were principally inhibited by magnesium salts.[145] A moderately growth-inhibiting effect was also ascribed to magnesium thiosulfate in mouse tumors,[146] and a similar observation was made in tar carcinoma of mice.[147] In the latter reports, the anion must also be considered of importance. This is clearly demonstrated by experiments according to which $MgSO_4$-injections, accompanying roentgen irradiation of Jensen rat sarcomas, enhanced tumor regression, whereas $MgCl_2$-injections unfavorably influenced the radiation therapy.[148]

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[136] P. Schrumpff-Pierron: Ztschr. f. Krebsforsch. *36*: 115 (1932).

[137] A. Sartory, R. Sartory, J. Meyer & E. Keller: Compt. rend. Soc. de biol. *195*: 400 (1932).

[138] R. Reding & A. P. Dustin: Compt. rend. Soc. de biol. *88*: 301 (1923).

[139] R. Reding: Compt. rend. Soc. de biol. *89*: 817 (1923).

[140] P. Delbet & C. Palios: Bull. Assoc. franç. p. l'étude du cancer *17*: 325 (1928).

[141] P. Delbet & C. Palios: Bull. Assoc. franç. p. l'étude du cancer *17*: 525 (1928).

The prevailing majority of investigators arrived at wholly divergent results. Peroral magnesium administration was seen as slightly promoting rat tumor growth,[149] and intravenous $MgCl_2$-injections acted similarly on rabbit sarcomas.[14] The growth of tumor explants was also enhanced by minimal magnesium concentrations.[150] A complete in-

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142. P. Delbet & C. Palios: Bull. Assoc. franç. p. l'étude du cancer *20*: 187 (1931).

143. M. Marullaz: Bull. Acad. de méd., Paris *102*: 48 (1929); *103*: 166 (1930).

144. A. Kotzareff, J. de Morsier & A. Morin: Bull. Acad. de méd., Paris *102*: 11 (1929).

145. A. H. Roffo: quot. A. H. Roffo & J. Thomas: La chimie du cancer, Vigot frères, Paris, 1933, p. 65.

146. J. Kabelik: Bratisl. Lekar. listy *8*: 409 (1928).

147. P. Estas: Rev. belg. sc. méd. *1*: 189 (1929); abstr. Cancer Rev. *5*: 15 (1929).

148. K. P. Markuse & D. A. Losinsky: Ztschr. f. Krebsforsch. *44*: 422 (1936).

149. K. Sugiura & S. R. Benedict: J. Cancer Research *7*: 329 (1923).

150. K. Uei: Fol. jap. pharmacol. *2*: 288 (1926); abstr. Chem. Zentralbl. *1*: 1616 (1927).

effectiveness of magnesium administration was observed in transplanted and in tar carcinomas of white mice,[151–154] in the tar carcinoma of rabbits [155] and in rat sarcomas.[156] Analogous negative results were obtained by other authors.[90, 157] A very exact study was performed on the Flexner-Jobling carcinoma of rats.[158] Here, in obvious contrast to Delbet's theories and reports, a magnesium-free diet was seen to impede tumor growth most strongly; after three weeks the weights of the tumors of treated animals reached only 4 per cent of the tumor weight of control animals fed on a normal diet. Addition of small magnesium amounts lessened this tumor inhibition, whereas large magnesium additions even accelerated tumor growth, and mouse tumors exhibited an analogous behavior. Although a reduction of the potassium calcium ratio, based on a calcium increase, was found in rat tumors following magnesium injections, this treatment did not influence the tumor beneficially; [129] on the contrary, an unfavorable effect on the general condition of the animals was observed.[159] In contrast to sodium, potassium and calcium (cf. above), magnesium did not increase the radiosensitivity of malignant tissue.[17, 131] Summarizing, it must be stressed that on the basis of the available experimental material, the assumption of an anti-tumoral quality for magnesium cannot be accepted. Consequently, it will not be surprising that in human tumor pathology, also, no convinc-

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151. R. Severi: Riv. di pat. sper. *8*: 327 (1932); abstr. Ztschr. f. Krebsforsch. *38*: Ref. 4 (1932).

152. L. Kreyberg & S. S. Nielsen: Acta path. et microbiol. Scandinav. *12*: 165 (1935).

153. F. M. Brikker: Libro de Oro, Buenos Aires, 1935, p. 134.

154. M. E. Brumpt: Bull. Acad. de méd., Paris *115*: 400 (1936).

155. P. Serbescu: Bull. Acad. de méd., Paris, *111*: 435 (1934); *117*: 101 (1937).

156. M. Cilotti: Pathologica *23*: 535 (1931).

157. S. Itami: J. A. M. A. *72*: 934 (1919).

158. K. Sugiura & S. R. Benedict: Am. J. Cancer *23*: 300 (1935).

159. A. Bolaffi: Tumori *16*: 420 (1930).

ing success was seen in the prophylactic or therapeutic use of magnesium. Older reports which claim a beneficial effect from such a treatment in epitheliomas [160, 161] are only of unsystematic character, and are restricted to the unreliable criteria of life prolongation and slower advancement of the process.[162, 163] The same applies to an alleged reduction of the tumor volume achieved by intramuscular $MgSO_4$—injections [164] and by magnesium-iontophoresis,[165] respectively. Later on, Delbet especially [166] advocated the use of a magnesium preparation (Delbiase) for purposes of cancer prophylaxis and for the prevention of postoperative recurrences without, however, being able to support this recommendation by convincing statistical and clinical material. The favorable effect ascribed by some authors [167–171] to the magnesium therapy employed in a few clinical experiments has not been confirmed by more critical investigations.[172, 173]

Reviewing the material discussed which, in spite of or perhaps because of its extensive scope, exhibits so many conflicting and confusing statements, one must agree with the opinion voiced by Shear [174] in a very carefully composed monograph. This author painstakingly and exhaustively

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160. J. Regnault: Bull. gén. ther. *168*: 737 (1915).
161. J. Regnault: Bull. Acad. de méd., Paris *79*: 205 (1918).
162. Dubard: Bull. Acad. de méd., Paris *79*: 205 (1918).
163. Dubard: Bull. Assoc. franç p. l'étude du cancer *8*: 148 (1919).; *9*: 370 (1920).
164. A. Slosse & R. Reding: Bull. Acad. Roy. méd. Belg. *4*: 268 (1924).
165. R. Reding & A. Slosse: Bull. Acad. Roy. méd. Belg. *5*: 166 (1925).
166. P. Delbet: Presse méd. *36*: 1473 (1928); *39*: 120 (1931).
167. A. Zimmern & Y. L. Wickham: Bull. Assoc. franç. p. l'étude du cancer *17*: 362 (1928).
168. H. Dufour: Presse méd. *39*: 120 (1931).
169. V. Riche: Arch. Soc. d. sc. méd. et biol. de Montpellier *12*: 77 (1931); abstr. Cancer Review *7*: 198 (1932).
170. R. H. Craig: Canad. M. A. J. *31*: 531 (1934).
171. R. P. Wright: Canad. M. A. J. *31*: 404 (1934).
172. G. Villata: Diario Radiol. No. 6 (1928).
173. G. Fichera: Chimioterapia del cancro, Ulric Hoepli, Milano, 1934.
174. M. J. Shear: Am. J. Cancer *18*: 924 (1933).

collected all pertinent publications (until 1933) and examined them critically, both with regard to the methods employed and the results obtained. He concluded that "our present knowledge of the part played by sodium, potassium, calcium and magnesium in cancer is incomplete and unsatisfactory. A study of the literature leaves one with the impression that sodium is of no especial significance, that potassium may have a stimulating effect, and that calcium appears to have a retarding effect on tumor growth No substantial evidence is found for the oft repeated assertion that magnesium has a beneficial effect in cancer

"So far as the mineral content of tumors is concerned, young, actively growing tumors appear to be richer in potassium than slowly growing or old tumors; the reverse appears to be the case with calcium. Not enough data have been obtained on magnesium and sodium contents of tumors to warrant generalization.

"So far as the inorganic composition of the blood is concerned, no relationship has been established between the cancerous process and the amount of sodium, potassium, calcium, or magnesium present in the blood serum (or plasma)."

Supplementing the discussion of the alkali metals, brief mention may be made of *caesium, lithium* and *ammonia.* Injections of caesium salts were found ineffective when administered to mice bearing Twort carcinomas, and a prophylactic treatment with this substance caused only slight inhibition of the tumor growth.[175] *Lithium* salts of trypan blue, carmine and other dyes have recently been utilized for increasing the effect of slow neutron radiation.[176, 177] The combination of lithium with the dyes resulted in achieving a concentration of this element in animal tumors which was twice as high as in the surrounding normal tissues. Hence the radiation effect on the tumor was increased by 43 per cent. An evaluation of this therapeutical attempt is not yet possible.

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[175] A. W. Wright & C. F. Graham: Am. J. Path. 9: 789 (1933).
[176] P. A. Zahl & F. S. Cooper: Science 93: 64 (1941).
[177] P. A. Zahl: Surgery 9: 327 (1941).

No characteristic difference was detected by a comparison of the ammonia content of malignant tumors and of normal organs[178] (cf. also paragraph on protein metabolism). Importance must be attributed to this negative finding, since thus the frequently quoted alkalosis of tumors (see chapter on physical chemistry) cannot be explained by an ammonia increase.

But little is known of the remaining alkaline earths—*barium* and *strontium*. From an analytical point of view it was reported that barium was found in the human body only in the choroid and in melanotic tumors.[179] Maisin and his collaborators,[180–182] on the basis of animal experiments, worked out a cancer therapy employing injections of ether extracts of brain, bone marrow and spleen, and at the same time peroral administration of minute amounts of barium, strontium and cadmium. The authors saw favorable effects from this treatment, principally in skin cancer and to a certain extent also in cancers of the oral cavity, when used in combination with radiation therapy.[183] A theoretical basis for the barium administration might be seen in the finding that barium salts increased the respiration of cancer tissue selectively.[184] But as no confirmatory studies of this treatment are available and, furthermore, since barium and strontium are used only as a supporting measure, the anti-tumoral quality of these elements cannot be considered as established. Claims of a beneficial therapeutic effect achieved in pulmonary metastases by injections of a 1 per cent barium nitrate solution [185] must be very critically evaluated, especially in view of the high toxicity of such a treatment.

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[178] A. Goldfeder: Ztschr. f. Krebsforsch. *38*: 147 (1932).

[179] W. Gerlach & R. Müller: Virchow's Arch. *296*: 588 (1936).

[180] J. Maisin & H. Vassiliadis: Compt. rend. Soc. de biol. *108*: 806 (1931).

[181] J. Maisin & C. Ronsse: Compt. rend. Soc. de biol. *110*: 1018 (1932).

[182] J. Maisin, H. Vassiliadis & E. Picard: Bull. Assoc. franç. p. l'étude du cancer *21*: 255 (1932).

[183] J. Maisin & H. Vassiliadis: Strahlentherapie *54*: 199 (1935).

[184] Y. Pourbaix: 1st Internat. Cancer Congress Madrid *1*: 72 (1933).

2. *Iron and Allied Elements.*

Iron. While an older statement [1] on the basis of a small number of experiments claimed a reduction of the iron content in tumors, no appreciable difference was found in the iron concentration in rat carcinomas if compared with normal epithelium.[186] This divergence could be due to a different connective tissue content of various tumors, since iron is mainly localized in the epithelium. Histochemical and microchemical determinations performed on a material comprising more than 170 human and animal tumors revealed a definite correlation of the iron content of the neoplasms with the iron content of the matrix.[187] Metastases generally proved to be poorer in iron than primary tumors. The increased iron content of irradiated tumors probably depends on vascular damage and blood disintegration. The same unspecific cause—presence of hemorrhages—was recognized as responsible for the increased iron content of fowl sarcomas.[188] To what degree this source of error was considered in a study reporting iron increase in the Ehrlich-Putnoky rat carcinoma is obscure.[189] An exact determination of the non-hemoglobin iron in tumors could prove of great value, insofar as this metal plays an important rôle in the cell respiration (Warburg's respiratory enzyme), and since the tumor respiration appears to differ from the norm (Cf. Chapter on metabolism). In contrast to the studies mentioned, later publications using newer methods found an iron decrease in some malignant tumors,[190, 191] but more extensive investigations must be awaited before definite conclusions can be made as to this problem and its bearing on the cancerous process.

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[185] K. Nemec: Med. Welt *9*: 1842 (1935).
[186] M. Yabusoe: Biochem. Ztschr. *157*: 388 (1925).
[187] S. Loewenthal & H. Probst: Ztschr. f. Krebsforsch. *42*: 222 (1935).
[188] V. Morávek: Ztschr. f. Krebsforsch. *36*: 573 (1932).
[189] J. Romhányi & M. Schmidt: Frankfurt. Ztschr. f. Path. *48*: 380 (1935).

As to the iron content of the body of the tumor host, in a paper mentioned above,[189] a diminution of the iron content was observed in the liver and spleen, an increase in the kidney, and a higher iron excretion in urine and feces of rats bearing Ehrlich-Putnoky carcinomas. All these findings suggest rather a connection with hemoglobin disintegration than a direct bearing on the tumor process. A normal ferric ion concentration was found in 6 analyzed sera of cancer patients,[192] and no difference of the iron composition was noted in the intestinal tract of normal and tumor-bearing rats.[193]

Comparatively few publications have been devoted to the experimental and chemotherapeutic use of iron, but beneficial results were seen in animal experiments of a colloidal iron preparation.[194] Intravenous injections of ferric saccharate, it was claimed, inhibited the growth of Brown-Pearce rabbit carcinoma[195] and of mouse carcinoma[196] by means of an oxidation increase. Injection of ferric gluconate impeded the growth of a transplantable rat sarcoma in one third of the animals. This effect was increased by simultaneous injection of neutral red or azo blue but resulted in complete

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190. S. G. Zondek & M. Bandmann: Deutsch. med. Wchnschr. *59*: 91 (1933).
191. K. Okamoto & T. Kira: Tr. Soc. path. jap. *26*: 161 (1936).
192. A. Langer: Biochem. Ztschr. *242*: 316 (1931).
193. H. Auler: Ztschr. f. Krebsforsch. *35*: 89 (1932).
194. B. Sokoloff: Compt. rend. Soc. de biol. *102*: 401 (1932).
195. H. Seel & H. Carls: Ztschr. f. Krebsforsch. *40*: 416 (1934).
196. G. Creutzberg & H. Seel: Strahlentherapie *42*: 684 (1931).

regression in only rare instances.[197] Iron and other metal compounds of oxidized ascorbic acid were found somewhat effective in animal tumors [198] but they did not prove beneficial in clinical experiments.[199] However, considering the results of Warburg,[200] who failed to find an increased oxidation of sea-urchin eggs after iron addition, it is questionable if, in the experiments mentioned, the iron was actually effective as catalyst for the oxidation processes or whether the action is not solely that of a colloid (cf. paragraph 8). In spite of the claim of the author,[201] little value can be attributed to a treatment employing injections of finely dispersed ferrosoferric oxide (Fe_3O_4) into skin cancers and subsequent application of electromagnetic radiation. The specific chemotherapeutic use of iron cannot be considered promising since in studies of neoplastic tissue cultures a growth promotion was noted following addition of iron salts.[150] On the other hand, the fact that three cases of pulmonary cancer were seen in persons exposed professionally to the inhalation of finely dispersed iron oxide dust [202] does not seem attributable to a specific carcinogenic action of this element, but it should rather be considered as an expression of a chronic mechanical irritation.

Manganese and cobalt. The presence of manganese was detected in minimal concentrations in transplanted tumors of mice, rats and dogs, probably not differing from amounts present in normal organs.[203] No analytical data are available as to cobalt. This element, however, evoked some interest in tumor pathology on account of the frequent observation of lung tumors in cobalt mine workers. The most familiar instance is the "Schneeberger pulmonary cancer" in Bohemia, but present evidence denies that cobalt is the causative agent and points, rather, to radioactive substances as being responsible.[204]

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[197] R. M. Brickner & R. E. Grant: Science *86*: 450 (1937).

[198] F. Arloing, A. Morel & A. Josserand: Compt. rend. Soc. de biol. *120*: 201 (1935); Compt. rend. Acad. d. sc. *202*: 598 (1936).

[199] F. Arloing, A. Morel & A. Josserand: Compt. rend. Acad. d. sc. *204*: 824 (1937).

[200] O. Warburg: Ztschr. f. physiol. Chem. *57*: 1 (1908).

[201] H. Spude: Ztschr. f. Krebsforsch. *13*: 139 (1913); *21*: 294 (1924).

[202] J. R. Dreyfus: Ztschr. f. klin. Med. *130*: 256 (1936).

[203] F. Medigreceanu: Proc. Roy. Soc., London, s. B *86*: 174 (1913).

Cobalt has been employed chemotherapeutically in mouse tumors in the form of dichloro-pentamminocobaltic chloride ($ClCo(NH_3)_5Cl_2$) [205] and analogous experiments have been performed with cobaltic salts.[206] The beneficial effect achieved by these substances, according to the authors, resembled the effect obtained by the heavy metals copper, silver, gold, etc. Essentially, the action was claimed to depend on an increase of autolytic processes in the tumor (cf. par. 8). Clinically, the injection of cobaltoxides into arteries near tumors was tried with moderate success only.[207]

3. *Earth Metals and Rare Earths.*

Aluminum. A carcinogenic quality has been ascribed to this metal [208, 209] but no analytical or experimental data were furnished to substantiate this claim. It was based rather on the completely arbitrary assumption that a relationship exists between the increased use of aluminum utensils and higher cancer mortality (the latter fact, incidentally, being a debated phenomenon). However, the value of this theory has been

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204. L. Carozzi: Seccion Social, 1st Internat. Cancer Congress, Madrid 1933.

205. W. Caspari: Ztschr. f. Krebsforsch. *14*: 236 (1914).

206. T. Ogata, M. Ishibashi, S. Kawakita & Y. Shibata: Tr. Jap. Path. Soc. *11*: 170 (1921).

207. E. Lauwers: Rev. belg. sc. méd. *5*: 377 (1933); abstr. Amer. J. Cancer *22*: 921 (1934).

208. C. T. Betts: Research Publishing Company, Toledo (Ohio) (1928); abstr. Chem. Zentralbl. *1*: 3006 (1929).

209. L. Spira: The clinical aspect of chronic poisoning by aluminum and its alloys, London, Bale, Sons and Dannielson Ltd. 1933; abstr. Wien. klin. Wchnschr. *47*: 1148 (1934).

generally refuted.[210-212] In this connection, a study may be mentioned which denied any biological importance of aluminum on the basis of feeding experiments to rats.[213] In further experiments, no resorption of aluminum was observed after a long period of administration to dogs and humans.[214] Finally, it was observed that the feeding of aluminum sulfate, on the contrary, tended to inhibit tumor development in rabbits painted with tar,[215] whereas the growth of the Kato rabbit sarcoma was not significantly influenced by intravenous or intramuscular injections of various aluminum compounds.[216]

Rare earths. The action of the rare earths—samarium, neodymium, lanthanum, yttrium, scandium—was systematically analyzed, and all were found to be ineffective.[217] Thallium, however, exhibited the property of provoking atypical growth of the mucous membrane of the forestomach of the rat.[218] On the other hand, this element has been employed for therapeutic purposes in combination with roentgen radiation (secondary radiation), and good results were claimed to have been achieved in skin, larynx and esophagus cancers.[219] The rationale underlying this treatment is probably the well known depilatory quality of thallium, i.e., a specifically epithelium-damaging effect. Thallium application, however, failed to benefit tumor-bearing mice [220] and clinically the

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210. G. Ichok: Ann. Hyg. Pub. N. S. *7*: 113 (1929); abstr. Chem. Zentralbl. *1*: 3006 (1929).
211. F. Blumenthal: Ztschr. f. Krebsforsch. *30*: 314 (1930).
212. K. B. Lehmann: Arch. Hyg. *102*: 349 (1929).
213. H. Steudel: Biochem. Ztschr. *253*: 387 (1932).
214. J. Wührer: Biochem. Ztschr. *265*: 169 (1933).
215. G. Bertrand & P. Serbescu: Ann. Inst. Pasteur *52*: 478 (1934); Compt. rend. Acad. d. sc. *198*: 1100 (1934).
216. T. Hamawaki: Jap. J. Obst. & Gynec. *23*: 284 (1940).
217. B. T. Simpson & M. C. Marsh: Ann. Surg. *93*: 169 (1934).
218. A. Buschke & B. Peiser: Ztschr. f. Krebsforsch. *21*: 11 (1924).
219. H. Auler: Ztschr. f. Krebsforsch. *23*: 153 (1926).
220. H. Hirschfeld: Ztschr. f. Krebsforsch. *16*: 33 (1919).

injection of thallium salts into arteries near tumors proved only moderately effective.[207] A general employment of this substance could, furthermore, not be considered advisable because of its highly toxic character.

Among the rare earths *cerium* has also been utilized in cancer therapy, viz., combined with iodine in the form of the preparation "Introcid," [221] in which iodine was supposed to act as vehicle for the metal (cf. on alleged tumor affinity of iodine, seq.). Subjective and clinical improvements of inoperable cancer cases were reported in a combinative therapy, recommended by Opitz,[222] (cf. Immunology), in which cerium chloride was used as a supporting measure. The oblivion to which these therapeutic attempts have succumbed may be considered a criterion of their small value.

Only ephemeral interest has been placed on the use of *rhenium.* A report on "cures" of a few cancer and sarcoma cases effected by intramuscular injections of potassium perrhenate [223] was entirely contradicted by a subsequently performed study, according to which the extensive use of this treatment failed to produce any beneficial effects in spontaneous and experimental mouse tumors. The treated animals even showed an increased mortality.[224]

4. *Silver Group (Copper, Silver, Mercury).*

Copper. As to the analytical data of the copper content of malignant tumors, in the course of time views expressed in the literature have undergone numerous changes. Even today a clear cut decision does not appear to have been reached. A constant difference between necrotic and non-necrotic tumor parts, with prevalence of copper in the first,

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221. C. Lewin: Ztschr. f. Krebsforsch. *27*: 132 (1928)
222. E. Opitz: Ztschr. f. Krebsforsch. *22*: 110 (1925).
223. S. -S. Fournier: Gaz. méd. de France 1936: 98.
224. N. Dobrovolskaia-Zavadskaia & A. Raymond: Compt. rend. Soc. de biol. *125*: 353 (1937).

seems to be the only well established fact. This was observed in an investigation comprising twenty-four human tumors and showing an average copper content of approximately 80 mg. per kg. of dried tissue, a value about equal to the copper concentration of spleen (78 mg./kg.) and exceeding that of liver (57 mg./kg.) and of kidney (58 mg./kg.).[225] Analyses of rat tumors yielded copper values markedly higher than those of other organs.[226] Although these papers do not support the assumption of a copper deficiency in tumors, as had been previously alleged and which has been made the basis of therapeutic attempts, more recently a diminution of this metal has been reported in human and animal tumors. The indirect copper determination of Warburg (catalysis of cysteine oxidation) was used in this study.[190] A reduced copper content of human neoplasms and of animal sarcomas was also claimed by another author.[227] A decreased copper concentration was also found in metastases-free livers of cancer patients,[228] although continued studies utilizing exact microchemical [229, 230] and spectrophotometric methods [231, 232] could not confirm a copper deficiency of human or animal tumors. Only the relative increase of copper in the necrotic tumor parts was again noted. This uncertainty as to the copper content of tumors is paralleled by data pertaining to the copper content of the cancer-affected organism. Whereas a progressing copper deficiency of the body was observed in Ehrlich-Putnoky rat carcinoma, and this fact was assumed to be responsible for the cancer

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225. C. P. White: Lancet *2*: 701 (1921).
226. I. Hieger: Biochem. J. *20*: 232 (1926).
227. M. Sugai: Mitt. a. d. med. Akad. zu Kioto *29*: 314 (1940).
228. Beck, abstr. G. Klein & W. Ziese: Ztschr. f. physiol. Chem. *213*: 201 (1932).
229. S. Sümegi: Frankfurt. Ztschr. f. Path. *48*: 35 (1935).
230. J. Putnoky & S. Sümegi: Ztschr. f. Krebsforsch. *41*: 505 (1935).
231. S. Edlbacher & W. Gerlach: Ztschr. f. Krebsforsch. *42*: 272 (1935).
232. W. Gerlach: Ztschr. f. Krebsforsch. *42*: 290 (1935).

anemia,[230] no change of the copper concentration was found in other animals or in patients suffering from cancer. Similarly, the problem of copper localization in tumors is still obscure; positive statements[231] conflict with negative ones.[226, 229] Admitting the possibility that these divergencies might be explained on the basis of differences in the analyzed tumors and the methods employed, no basis at present appears to exist for ascribing a primary role to copper in the tumor problem, or for correlating with tumor pathology the known physiological copper functions in growth and metabolism.

Investigations of the copper level of the blood gave no characteristic results.[233] The normal average value of 0.94 mg. per cent is increased to 1.45 mg. per cent in secondary anemias, but cancer patients and luetics, in whom the anemia is not accompanied by increased blood formation, show values reduced to 0.5–0.7 mg. per cent.

Mainly independent of the analytical results, therapeutic attempts using copper were initiated long ago. As early as 1913 beneficial effects achieved in human cancer by intravenous injections of colloidal copper solutions were reported, although only slow-growing tumors responded favorably.[234] A tumor inhibiting effect of copper was seen in animal experiments both after injection[205, 226, 235] and after peroral administration,[231, 236] and this was confirmed by means of cancer tissue cultures.[150] Further successes of copper therapy were reported;[237] the intramuscular injection of colloidal copper combined with X-ray therapy was considered especially promising.[238] Gaube du Gers,[239] employing a colloidal copper-aluminum preparation ("Cuprase"), reported on 50 cancer cases in which this treatment allegedly produced cures or far-reaching improvement. Favorable experiences were also obtained with a copper-alanine compound.[240] The objection may be raised, however, against these claimed successes in that histologic confirmations were generally lacking, and the time of observation was too short. The results obtained by Fichera [178] were unsatisfactory: In 20 cases subjected to copper therapy he observed strong local reactions, sometimes slight improvements, but cures were never achieved. In view of the specific toxic effect of copper on the reticulo-endothelial system,[241] the author even warned against its use in cancer therapy. The lacking therapeutic effect of copper on tumors was asserted also by several other authors.[242-245]

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[233] C. Bence: Wien. klin. Wchnschr. *48*: 1075 (1935).

[234] L. Loeb, C. B. McClurg & W. O. Sweek: Am. J. Pharmacol. *85*: 190 (1913).

[235] A. J. Gelarie: Brit. med. J. *2*: 222 (1913).

[236] K. Sugiura & S. R. Benedict: J. Cancer Res. *7*: 329 (1923).

[237] M. Mannelli: Rass. clin. *28*: 43 (1929); abstr. Chem. Zentralbl. *1*: 3006 (1929).

Mercury. The influence of this element on neoplasms appears to be insignificant. Although one author [246] described necroses, hemorrhages, reduction in size and sometimes regression of animal tumors following mercury treatment, the results of another study were entirely negative, regardless of the form of application (peroral, percutaneous, subcutaneous).[247]

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238. de Nabias: Bull. Assoc. franç. p. l'étude du cancer *19*: 343 (1930).

239. Gaube du Gers (1913), abstr. R. Cruickshank: Cancer Rev. *6*: 537 (1931).

240. J. Donelan: Lancet *197*: 1107 (1919); *198*: 501 (1920).

241. N. von Jancsó: Ztschr. f. d. ges. exper. Med. *56*: 135 (1927); Klin. Wchnschr. *10*: 537 (1931).

242. R. Weil: J. A. M. A. *61*: 1034 (1913); *64*: 1283 (1915).

243. H. Roziès: Gaz. d. hôp. *86*: 327 (1913); abstr. Wien. klin. Wchnschr. *26*: 822 (1913).

244. G. Burzi: Gaz. degli osped. 1913, No. 119; abstr. Wien. klin. Wchnschr. *27*: 57 (1914).

245. W. A. Collier & F. Krauss: Ztschr. f. Krebsforsch. *34*: 526 (1931).

246. H. R. Gaylord: Berlin. klin. Wchnschr. *49*: 2017 (1912).

247. S. Skudro: Wien. klin. Wchnschr. *26*: 577 (1913).

Silver. The studies concerned deal with therapeutic attempts which must be evaluated in a negative sense.[173] Intravenous injection of colloidal silver preparations (collargol, electrargol, fulmargan) were used for the most part[248, 249] and violent general reactions (nausea, cyanosis, fever) were observed. The use of insoluble silver salts does not appear to be protection against these complications. At least in animal experiments (mice) the administration of silver chloride-emulsions was followed by the severest toxic phenomena, causing the death of the animals, although the tumors showed liquefaction.[250] A unique but, scientifically, only slightly valuable experiment was performed, giving homeopathic doses of silver nitrate (10^{-11}—10^{-15} Gm.) to mice affected with tar cancer.[251] Only these minute silver amounts were claimed to result in a cure, while greater doses were reported to stimulate tumor growth. Such a growth-promoting effect was seen also in Flexner-Jobling rat carcinoma, following intratumoral injection of colloidal silver.[252]

5. *Gold and Platinum.*

In addition to the older studies of Neuberg and Caspari,[253] mentioned below, C. Lewin[254] devoted considerable work to cancer therapeutic experiments with *gold.* He used, principally, the compound potassium aurocyanate ($K(Au(CN)_2)$) and achieved hemorrhage, liquefaction and reduction of volume of transplanted mouse tumors by means of its intravenous injection. Reports on beneficial clinical effects and

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[248] G. L. Rohdenburg: J. med. Res. *26*: 331 (1915).

[249] W. S. Stone, G. T. Pack & H. Q. Woodard: Ann. Int. Med. *3*: 149 (1930).

[250] O. Backofen & S. Ikeda: Ztschr. f. Krebsforsch. *39*: 308 (1933).

[251] L. E. Walbum: Ztschr. f. Krebsforsch. *31*: 1 (1930).

[252] M. F. Guyer & F. E. Mohs: Arch. Path. *15*: 796 (1933).

[253] C. Neuberg & W. Caspari: Berl. klin. Wchnschr. *49*: 1405 (1912).

[254] C. Lewin: Berl. klin. Wchnschr. *50*: 541 (1913).

"cancer cures," however, achieved by peroral and parenteral administration of a commercial gold preparation [255, 256] must be viewed with considerable scepticism, as they are based on a few cases only, and some of them were not confirmed histologically. This therapy was also claimed to have accomplished the disappearance of one single spontaneous rabbit tumor.[257] According to Heubner,[258] who dealt extensively with the pharmacology of gold, this metal possesses a capillary damaging quality which caused doubt as to the desirability of its therapeutic use.[173] On the basis of Heubner's conception, Lewin assumed that the effect of gold on tumors might be explained by a damage to the tumor nutrient vessels, thus producing a severe nutritional disturbance of the neoplasm which, a priori, has been insufficiently vascularized.

Platinum. This metal, as well as many others, was injected intravenously into tumor-bearing mice by Neuberg and Caspari [253] in the form of complex organic compounds, and tumor destruction caused by this treatment was noted. Regressions of Flexner-Jobling rat carcinomas observed after intratumoral platinum injections [252] do not warrant definite conclusions as to the chemotherapeutic value of this metal.

6. *Heavy Metals: Chromium, Tin; Molybdenum, Tungsten; Zinc, Cadmium; Bismuth, Lead.*

The presence of *chromium* was determined in carcinomas and in sarcomas, but also in fibromas and myomas, in amounts up to 0.25% of the ash.[259–261] An evaluation of these findings

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[255] E. H. Ochsner: Illinois Med. J. (July) 1926; Internat. J. Med. & Surg. *40*: 100 (1927); Clin. Med. & Surg. (July) (1935).

[256] L. H. Nowack: Wisconsin Med. J. *25*: 139 (1926).

[257] G. E. Jorgenson: Clin. Med. & Surg. (January) 1929.

[258] W. O. L. Heubner: Arch. f. exper. Path. u. Pharmakol. *56*: 370 (1907).

[259] A. Dingwall & H. T. Beans: Am. J. Cancer *16*: 1499 (1932).

[260] A. Dingwall & H. T. Beans: Proc. Nat. Acad. Sc. *20*: 416 (1934).

[261] A. Dingwall, R. G. Crosen & H. T. Beans: Am. J. Cancer *21*: 606 (1934).

and their importance for the cancer problem is not possible for the time being. The frequent occurrence of pulmonary cancer in workers occupied in the chromium industry has been observed by several authors. The first reports [262, 263] viewed a causal connection with great reservation. But since additional publications have reported respectively seven [264] and fifteen cases [265] of bronchiogenic cancer occurring in workers in chromate factories, this high tumor incidence made a carcinogenic quality of chromium rather probable. According to German insurance law, pulmonary cancer of chromium workers has been recognized as an occupational disease.[266] This problem still awaits elucidation by means of animal experiments testing the carcinogenic activity of chromium. In this respect, only an unsuccessful attempt has been recorded; no lung tumors could be produced in mice by means of a single chromite injection, nor did this substance influence the tumor production by methylcholanthrene.[267] Nevertheless, cautious judgment must be applied to chemotherapeutic experiments in which intravenous, subcutaneous or peritumoral injections of CrO_3-solutions were used in combination with radiation therapy; moderately beneficial results have been claimed.[268]

Tin in the form of complex organic compounds proved especially effective when injected intravenously into tumor-bearing mice, according to the already quoted studies of Neuberg and Caspari.[253]

Molybdenum has been detected in 17 out of 59 analyzed

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[262] K. B. Lehmann: Zentralbl. f. Gewerbehyg. *9*: 168 (1932).

[263] Koelsch: Arch. f. Gewerbepath. u. Gewerbehyg. *3*: 454 (1932).

[264] E. Pfeil: Deutsche med. Wchnschr. *61*: 1197 (1935).

[265] W. Alwens, E. E. Bauke & W. Jonas: Arch. f. Gewerbepath. u. Gewerbehyg. *7*: 69 (1936).

[266] E. Holstein: Med. Klin. *33*: 329 (1937).

[267] M. B. Shimkin & J. Leiter: J. Nat. Cancer Inst. *1*: 303 (1940).

[268] Grode: Strahlentherapie *55*: 462 (1936).

breast tumors.[260] The significance of this finding for cancer pathology still remains obscure. According to a single communication, compounds of molybdenum and *tungsten* respectively, (sodium molybdenate, sodium phosphomolybdenate, sodium tungstate) exert a hypoglycemic and oxidation-increasing influence. For this reason five cancer patients were treated with subcutaneous injections of sodium tungstate. An absence of toxic effects with only moderate therapeutic success was observed, as expressed in relief from pain, lessening of exudation, and gradual reduction of tumor size.[269] The number of cases tested was small and histologic data are lacking.

Zinc. Particular interest has been focused on this element in the tumor literature, since a strikingly high zinc concentration was found to be present in neoplasms.[270] In tumors of predominantly cellular structure a zinc content up to 0.1% of the dried substance was found, whereas only traces of this element are detectable in normal organs. This phenomenon was correlated with the increased mitotic activity, i.e., with the increased growth of tumors. Actually, an increased zinc resorption and storage was also observed in normal rats during the period of growth.[271] Young rats kept on a zinc-deficient diet showed a growth retardation, which was replaced by normal growth after zinc addition to the diet.[272] Analyses of human and animal tumors confirmed the increased zinc content [273, 274] and the relationship to the increased growth rate was made probable on the basis of similar findings in embryonic tissue.[273] A moderate increase of the zinc level was found in the serum and urine of tumor patients, but was not considered specific, as similar results were obtained in inflammatory processes.[275]

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[269] H. N. Mukherjee: Calcutta Med. J. *30*: 452 (1936).

[270] P. Cristol: Bull. Soc. chim. biol. *5*: 23 (1923).

[271] L. T. Fairhall: J. Biol. Chem. *70*: 495 (1926).

[272] H. G. Day & E. V. McCollum: Proc. Soc. Exper. Biol. & Med. *45*: 282 (1940).

[273] A. H. Roffo & J. Lasserre: Bol. inst. de med. exper. para el estud. y trat d. cáncer *1*: 616 (1925).

[274] M. Sugai: Mitt. a. d. med. Akad. zu Kioto *21*: 1298 (1937).

A theory of a highly complex nature assumed that the zinc augmentation in cancer represented a defensive reaction of the body "to counteract the increased colloidal activity of cancer tissue" and particularly "to inhibit the increased influence of proteolytic enzymes and to stimulate the peroxidases, respectively." [276] But these assumptions certainly should be supported by experimental evidence. In this respect it was reported only that zinc seems to inhibit yeast fermentation.[277] To what extent the recently ascertained constant zinc content of carbonic anhydrase,[278, 279] an enzyme important for the oxidative metabolism, is connected with the problem under discussion cannot be decided pending experimental clarification. Another purely speculative theory constructed an antagonism between cancer and tuberculosis incidence on the basis of an alleged zinc deficiency in the latter disease.[280]

A complex mechanism appears to be involved in a tumor producing action of zinc salts; that is, in the case of teratomas which developed in fowl testis following local injection of zinc chloride.[281] Only during the first three months of the year could this result be obtained. The conclusion that hormonal factors are responsible for this was proven by the simultaneous administration of gonadotropic hormones. Under these conditions the teratoma production was increased and also made possible at other seasons.[282] Negative results of teratoma formation by zinc chloride [283] can be explained by the neglect of these seasonal-hormonal factors. In continued studies the tumor producing activity of zinc chloride in fowl testis was confirmed and both a rapid penetrating growth of the teratomas and their transplantability were observed.[284] Zinc sulfate proved to be as effective as zinc chloride, thus showing the anion to be indifferent.[285] The zinc action was considered to cause a partial castration; the subsequent excessive regeneration during the season of increased spermatogenesis should then result in tumor formation.[286] The teratoma character of the tumor was explained with the pluripotential anlagen of the gonocytes from which the tumors are derived.[287] Recently a similar effectiveness of copper sulfate in producing fowl teratomas was reported,[288] which makes it probable that an unspecific metal effect is involved. That zinc salts do not possess a general carcinogenic property was shown in experiments using a mouse strain of a high-mammary tumor incidence. Injections of zinc sulfate into the breast tissue did not promote cancer formation but, on the contrary, delayed tumor appearance to a significant degree.[289] Similarly, zinc sulfate injections into the thymus of a mouse strain of high thymus tumor in-

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[275] M. Sugai: Mitt. a. d. med. Akad. zu Kioto *22*: 387 (1938).

[276] A. Zlataroff: Bull. Soc. chim. biol. *12*: 44 (1930); Wien. med. Wchnschr. *83*: 46 (1933).

[277] A. Zlataroff, M. Andreitschewa & D. Kaltschewa: Biochem. Ztschr. *231*: 123 (1931).

[278] D. Keilin & T. Mann: Biochem. J. *34*: 1063 (1941).

[279] E. Hove, C. A. Elvehjem & E. B. Hart: J. Biol. Chem. *136*: 425 (1941).

[280] D. B. Cruickshank: Tuberculosis, Cancer and Zinc. London, Medical Publications, Ltd. 1936.

[281] I. Michalowsky: Virchow's Arch. f. path. Anat. *267*: 27 (1928); *274*: 319 (1929).

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282. H. J. Bagg: Am. J. Cancer *26*: 69 (1936).
283. G. Kahler: Frankfurt. Ztschr. f. Path. *50*: 281 (1937).
284. V. Anissimova: Am. J. Cancer *36*: 229 (1939).
285. L. I. Falin & K. E. Gromzewa: Am. J. Cancer *36*: 233 (1939).
286. Ch. Champy & J. P. Lavedan: Bull. Assoc. franç. p. l'étude du cancer *28*: 503 (1939).
287. L. I. Falin: Am. J. Cancer *38*: 199 (1940).
288. L. I. Falin & W. W. Anissimova: Ztschr. f. Krebsforsch. *50*: 339 (1940).
289. F. Bi[illegible] & M. L. Long: Am. J. Cancer *37*: 531 (1939).

cidence failed to influence the tumor development.[290] On the basis of but few experiments using a transplantable rabbit sarcoma, a tumor inhibiting quality has been ascribed to zinc.[291] A different, entirely unspecific effect is involved in the use of zinc chloride for cauterizing purposes of uterine carcinoma [292, 293] and also of breast cancer.[294]

In tissue cultures zinc salts [150] as well as *cadmium* salts [295] impeded growth of cancerous tissue. With regard to cadmium, its use in the combinative chemotherapy of Maisin [180–183] has been mentioned above.

Bismuth. The quite substantial literature referring to the place of bismuth in tumor pathology is based principally on the assumption that it has a strong affinity for tumor tissue.[296, 297] Although no selective localization in tumors was claimed, nevertheless objections in conflicting reports [298–301] were brushed aside because their findings were declared to be based either on errors in method,[298] or because of deviations of the dosage used (too large, and toxic amounts), or because of differences in the time of analyses (too short intervals following injection), or, finally, because of the histologic structure of the tumors (advanced necroses, increased content of connective tissue). It is clear from these numerous secondary factors mentioned that the bismuth localization in

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290. F. Bischoff & M. L. Long: Am. J. Cancer *38*: 404 (1940).
291. M. Sugai: Mitt. a. d. med. Akad. zu Kioto *29*: 315 (1940).
292. V. Czerny: Arch. f. klin. Chir. *61*: 287 (1900).
293. Sims & van de Wacker; Fränkel, quot. C. Lewin: Ergebn. d. inn. Med. u. Kinderh. *8*: 499 (1912).
294. C. W. Strobell: Clin. Med. & Surg. *42*: 173 (1935).
295. A. H. Roffo & O. Calcagno: Ztschr. f. Krebsforsch. *34*: 1 (1931).
296. H. Kahn & H. Wirth: Klin. Wchnschr. *6*: 2335 (1927).
297. H. Kahn: Strahlentherapie *37*: 151 (1930).
298. L. Califano: Ztschr. f. Krebsforsch. *26*: 183 (1928).
299. A. Lacassagne & J. Loiseleur: Compt. rend. Soc. de biol. *107*: 412 (1931).
300. A. Lacassagne: Compt. rend. Soc. de biol. *107*: 458 (1931).
301. K. Overhof: Strahlentherapie *54*: 261 (1935).

tumors has not yet been established as a general occurrence. Nevertheless, a chemotherapeutic effectiveness of bismuth was found in rat carcinomas.[302] Of the bismuth treatment of human neoplasms at least temporary improvements were reported even in advanced cases.[303] It was especially stressed that radioresistant tumors could be made radiosensitive by this treatment and irradiated tumor cells were found also to show a greater ability for storing bismuth.[304] A combination of bismuth and its isotope radium E has been said to be especially effective. By bringing a radioactive element directly to the tumor cell the same effect was supposed to be obtainable as by the use of combined injections of inactive bismuth and radiation therapy (cf. paragraph on radioactive elements). However, intratumoral injection of colloidal bismuth into Kato rabbit sarcoma was followed by an increased radiosensitivity, but a toxic vascular damage was recognized as the unspecific cause of this phenomenon.[305] Little validity can be assigned to an explanation of the bismuth therapy based on its supposed neutralization of the lactic acid produced by the tumor,[306] especially as today the previously assumed importance of lactic acid for tumor proliferation is negated (cf. chapters on organic chemistry and physical chemistry).

The insufficient rationale of the bismuth therapy is reflected in the unfavorable results derived from re-investigations of clinical material [307-309] and from animal experiments.[300]

Lead. The property of storing lead selectively has for a

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302. F. Ishiwara: Ztschr. f. Krebsforsch. *21*: 268 (1924).
303. H. Kahn: Deutsch. med. Wchnschr. *56*: 2131 (1930).
304. G. Janson: Acta radiol. (Stockholm) *13*: 533 (1932).
305. T. Kikuchi: Jap. J. Obst. & Gynec. *19*: 33 (1936).
306. P. Zadik: Deutsche med. Wchnschr. *56*: 826 (1930); Ztschr. f. Krebsforsch. *35*: 78 (1932).
307. F. Keller: Zentralbl. f. Gynäk. *57*: 1859 (1933).
308. F. Lasch & A. Neumann: Klin. Wchnschr. *8*: 1021 (1929).
309. R. Herbst: Klin. Wchnschr. *8*: 2049 (1929).

long time been ascribed to tumor tissue. In particular, Blair Bell[310] assumed that the alleged augmented lecithin content of tumors was responsible for the lead localization, by formation of a lead-lipid compound. He and his collaborators[311] in analyses of tumors and surrounding normal tissues found a four to ten times greater lead content in the former, after lead administration. The claimed affinity of lead for lipids induced Bell to initiate chemotherapeutic experiments, guided by the idea that important nutritional material could thus be withheld from the neoplasms and, consequently, their growth be inhibited.[312] However, not only these therapeutic attempts, but also their presupposition, the augmented lead localization in tumors, encountered numerous well founded objections. Thus, several authors[313, 314] did not detect lead in tumors following intravenous injection of various lead compounds, or did not observe an increased tumor affinity for this metal.[315-317] It has been stated repeatedly[173, 317, 318-321] that lead localizes mainly in the kidney, liver, bone marrow and spleen, i.e., in the reticulo-endothelial organs. The significance of this finding will be discussed later.

As to the therapeutic results, a publication of Bell[322] summarized *227* cancer cases subjected to lead therapy. A com-

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310. W. B. Bell: Lancet *2*: 1005 (1923).
311. M. Jowett, W. J. Dilling & W. B. Bell: Lancet *1*: 126 (1929).
312. W. B. Bell: Lancet *209*: 1003 (1925).
313. M. C. Reinhard & K. W. Buchwald: J. Cancer Res. *13*: 239 (1929).
314. B. T. Simpson: Intern. Conf. Cancer, London 1928: 208.
315. N. Kawata: Beitr. z. path. Anat. u. z. allg. Path. *82*: 259 (1929).
316. G. M. Antonioli & G. Villata: Arch. di farmacol. sper. *50*: 268; 271 (1930).
317. E. Flint, P. L. Günther & F. Eichholtz: Arch. f. exper. Path. u. Pharmakol. *169*: 618 (1933).
318. H. S. Martland & S. A. von Sochocky: J. A. M. A. *88*: 911 (1927).
319. W. J. Dilling: Internat. Conf. Cancer, London 1928, 212.
320. A. P. Thomson: Internat. Conf. Cancer, London 1928, 221.
321. T. Kikuchi: Jap. J. Obst. & Gynec. *18*: 88 (1935).
322. W. B. Bell: Brit. M. J. *1*: 431 (1929); *2*: 63 (1930).

plete arresting of the disease was accomplished in ten patients, thirty-one patients appeared to be cured, and the treatment was then discontinued. Fifty-one patients died prior to, and 106 died after, completed treatment; among the latter were two deaths due to acute nephritis caused by lead intoxication. These results may still be considered justification for the employment of the lead therapy insofar as a certain percentage of inoperable cancer patients showed improvement and even cures. However, this optimistic view is confronted by the fact that in numerous re-examinations a beneficial effect of this therapy on the tumor process was found only in a very few cases,[323–331] whereas most studies [173, 319, 332–346] arrived at completely negative conclusions as to the value of

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323. W. A. Collier: Ztschr f. Hyg. *110*: 169 (1929).
324. W. A. Collier & F. Krauss: Ztschr. f. Krebsforsch. *34*: 526 (1931).
325. E. J. Wang, P. T. Watson & J. A. Curran: Chin. med. J. *44*: 370 (1930).
326. L. Cunningham: Brit. M. J. *2*: 931 (1926).
327. P. Meyer: Klin. Wchnschr. *7*: 215 (1928).
328. E. Talbot: Brit. M. J. *2*: 1034 (1928).
329. M. M. Datnow: Monatschr. f. Geburtsh. u. Gynäk. *80*: 243 (1928).
330. M. Fournier: Presse méd. *38*: 1455 (1930).
331. A. E. Osterberg, B. T. Horton, J. A. Bargen & F. Rankin: Proc. Staff Meet. Mayo Clin. *7*: 231 (1932).
332. J. Lavedan: Paris. méd. *1*: 272 (1929).
333. A. Levi & E. Volta: Arch. di biol. *7*: 25 (1930).
334. R. Arrigoni: Arch. biochim. ital. *2*: 555 (1930).
335. O. Schürch: Deutsche med. Wchnschr. *59*: 1494 (1933).
336. A. H. Roffo & O. Calcagno: Prensa méd. Argent. *15*: 941 (1929).
337. Cesa-Bianchi: Atti soc. ital. med. Parma (1929); quot. 173.
338. M. Kochmann: Klin. Wchnschr. *7*: 1646 (1928).
339. A. Kaemmerer: Deutsche med. Wchnschr. *54*: 138 (1928).
340. S. Wyard: Brit. M. J. *1*: 838 (1928).
341. J. B. Hume: Internat. Conf. Cancer, London 1928, 239.
342. G. Loewy & J. Loiseleur: Internat. Conf. Cancer, London 1928, 246.
343. H. J. B. Fry: Internat. Conf. Cancer, London 1928, 250.
344. A. Mattina: abstr. Cancer Rev. *7*: 197 (1932).
345. Dalimier & Schwartz: 1st Internat. Cancer Congress, Madrid, *2*: 858 (1933).
346. J. C. Aub, & R. H. Smithwick: New England J. Med. *208*: 310 (1933).

the therapy. Several authors,[347-351] furthermore, reported on severe damage of the erythropoietic apparatus and of the kidneys (anemia, icterus, hematuria, nephritis) caused by the treatment. The interpretation of some favorable results is made difficult by the simultaneous use of radiation therapy. But this combined treatment also led to conflicting claims; besides beneficial effects,[352-359] negative reports [360-362] were offered. In this connection, the following experiments are significant; in normal cats the administration of colloidal lead caused much severer toxic symptoms when combined with roentgen radiation.[363] This finding certainly must evoke caution as to the clinical use of such a treatment, although the following observations must also be considered. Various soluble and insoluble colloidal lead compounds were tried by different authors, guided by the idea of preferring lead compounds of high metal content and low toxicity. This trend, however, is not completely justified, as some authors [173, 331] expressed the opinion that lead therapy was more effective

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347. R. W. Brookfield: J. Path. & Bact. *31*: 277 (1928).
348. S. Dentici, A. Moratti & P. Pattarin: Tumori *15*: 663 (1929).
349. E. Polichetti: Rinasc. med. *6*: 361 (1929); abstr. Cancer Rev. *6*: 129 (1931).
350. B. F. Schreiner & R. C. Wend: Surg., Gynec. & Obst. *48*: 115 (1929).
351. S. E. Gould, H. J. Kullman & H. A. Shecket: Am. J. M. Sc. *194*: 304 (1937).
352. F. Coke & J. B. Cook: Brit. M. J. *1*: 415 (1926).
353. H. J. Ullmann: J. A. M. A. *89*: 1218 (1927).
354. F. C. Wood: J. A. M. A. *89*: 1216 (1927).
355. J. C. Mottram: Brit. M. J. *1*: 132 (1928).
356. L. C. Knox: J. A. M. A. *92*: 106 (1929).
357. A. Brunner: Schweiz. med. Wchnschr. *59*: 235 (1929).
358. E. Duroux: Bull. méd. Paris *45*: 797 (1931).
359. J. A. Bargen, B. T. Horton & A. E. Osterberg: Am. J. Cancer *23*: 762 (1935).
360. A. T. Todd & H. M. Aldwinkle: Brit. M. J. *2*: 799 (1929).
361. C. A. Waters, J. A. C. Colston & L. N. Gay: J. A. M. A. *92*: 14 (1929).
362. A. Soiland, W. E. Costolow & O. N. Meland: J. A. M. A. *92*: 104 (1929).
363. B. L. Crawford, H. L. Stewart, C. E. Willoughby & F. L. Smith: Am. J. Cancer *33*: 40 (1938).

when subtoxic doses were employed. The experiences collected by Fichera[173, 331] did not demonstrate a definite value of the lead treatment applied to fifty-five patients. The more favorable results of Bell could be confirmed only as far as gynecologic cancers responded far better to the treatment than tumors of other parts. The cancer curative property ascribed to a commercial lead ointment ("tumorsan")[364, 365] cannot be considered as scientific evidence, because of the small and uncritically analyzed material.

As to the mechanism of the lead action in the successfully treated cases, Fichera[173] viewed a stimulation of the reticuloendothelial system as being responsible. Actually, as mentioned above, this system was found to localize lead in large amounts. Furthermore, an increased functional activity of this system was made probable, as expressed by an increased number of circulating monocytes following lead administration.[366] Another author[367] considered the vascular apparatus of the tumor connected with the lead action (cf. paragraph on metal action).

However, a decisive objection against the rationale of the lead therapy was based on the observation that persons occupationally exposed to lead are in no way protected against cancer.[368] On the contrary, inhalation of lead dust appeared to increase the frequency of lung tumors in exposed persons.[369] A recent study, however, indicated that the available statistical material was insufficient to permit the drawing of definite conclusions as to a greater or lesser cancer incidence among workers exposed to lead.[369a]

As to animal experiments, a growth inhibition of Rous

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364. Fronz; Micholitsch: Med. Rundschau 1935, No. 236.
365. R. L. Braun: Wien. klin. Wchnschr. *49*: 796 (1936).
366. Bianchini, abstr. Fichera l. c. 173.
367. F. C. Wood: Brit. M. J. *2*: 928 (1926).
368. G. B. Da Bona: Med. del Lavori *20*: 21 (1929/30).
369. F. H. Müller: Ztschr. f. Krebsforsch. *49*: 57 (1939).
369a. B. J. Black: J. Missouri M. A. *37*: 298 (1940).

sarcoma by lead-undecylenate was observed,[370] and fowls seemed to be protected by a lead-sulfur-pyridine-compound against subsequent Rous sarcoma inoculation.[371] The complex character of these compounds makes it appear possible that the lead alone was not necessarily responsible for these effects, and this might explain the fact that several other authors [372–377] failed to notice any effect from lead application in spontaneous, transplantable and tar tumors. The administration of a lead-trypan-blue compound did not affect the development of tar cancer in mice, but markedly decreased the frequency of metastases in regional lymph nodes.[378] A growth inhibition of Kato rabbit sarcoma, after intramuscular injections of colloidal lead, has been attributed to a stimulation of the reticulo-endothelial function determined by the congo red test performed before and after the treatment.[379] Animal experimentation using various lead compounds gave favorable results with lead salts of the following substituted amino acids: benzene sulfonylglycine ($C_6H_5SO_2.NH.CH_2COOH$), p-iodobenzene sulfonylglycine ($I.C_6H_4.SO_2.NH.CH_2COOH$), toluene-*2*:4-disulfonylglycine ($CH_3.C_6H_3(SO_2NH.CH_2COOH)_2$). o-thiolbenzoic acid.[380]

7. *Radio-active Elements.*

There is no intention to deal with the field of radio-therapy of malignant tumors in this place, despite the fact that this method is the only one, besides surgery, enjoying general

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370. N. Waterman: Ergebn. d. inn. Med. u. Kinderh. *30*: 304 (1926).
371. P. Zadik: Klin. Wchnschr. *12*: 1145 (1927).
372. B. T. Simpson: Intern. Conf. Cancer, London, 1928, 244.
373. H. E. Eggers: Arch. Path. *18*: 507 (1934).
374. C. Krebs & J. Clemmesen: Ztschr. f. Krebsforsch. *41*: 260 (1935).
375. P. L. Woodhouse: Am. J. Cancer *27*: 285 (1936).
376. H. Schmidt & G. Domagk: Medizin & Chemie III, 418 (1936).
377. W. A. Selle, F. Paquin & P. Brindley: Am. J. Cancer *38*: 86 (1940).
378. S. Bursell: Acta path. et microbiol. Scandinav. *18*: 1 (1941).
379. H. Kawaguchi: Jap. J. Obst. & Gynec. *23*: 178 (1940).
380. M. Datnow & others: Am. J. Cancer *24*: 531 (1935).

appreciation on the basis of its accomplishments. Readers interested in this field may be referred to text books on the subject and to periodicals on radiation therapy. The discussion here must be confined to reports of a general nature and to experimental data within the same limits as observed in previous sections.

Because of its originality, mention will be made of a theory which aims to explain the effect of radium and also of roentgen radiation by a cleavage of choline from the lecithin molecule caused by these radiations. The increased radiosensitivity of malignant tumors was ascribed to their augmented lecithin content.[381] Although the validity of this theory cannot be maintained, it gave rise to certain therapeutic experiments discussed in the chapter on organic chemistry.

Radium. It was claimed in an earlier report that the presence of radioactive substances was detected in several primary and metastatic tumors which had not been subjected to radiation therapy.[74] No further experiments, however, have been reported on this question since this study which was performed by old and inadequate methods. Theories alleging a causal connection between radioactivity and carcinogenesis [73, 74] may be referred to here and, at the same time, it may be stressed that experimental evidence, dealing with radioactive potassium, did not support such an assumption.[75] Although, in accordance with this it appears wholly impossible to hold radioactive substances generally responsible for the cancer etiology, a carcinogenic quality of radium and allied substances must be considered as definitely established—not only by clinical experiences but also from the results of animal experimentation. In the latter respect numerous authors [382-390] produced malignant tumors by means of radium

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381. R. Werner: Strahlentherapie *1*: 442; 452 (1912).

382. F. Daels: Bull. Assoc. franc. p. l'étude du cancer *15*: 102 (1926).

383. F. Daels & R. Biltris: Bull. Assoc. franc. p. l'étude du cancer *20*: 32 (1931).

application to rabbits, guinea pigs, rats, mice and fowl. Osteosarcomas were produced in rabbits by mesothorium,[387] transplantable sarcomas in mice and rats by intraperitoneal injection of thorium dioxide,[391-395] and breast tumors arose in guinea pigs after local thorotrast injections.[396] As to human pathology, it is well known that precancerous skin lesions as well as true cancers are frequently observed in persons occupationally exposed to radium (physicists, radio-therapists, radium dial painters).[396a, 397, 398] A few cases of malignant neoplasms also belong in this category, which developed after therapeutic radium application (female sex organs,[399] tibia).[400] The old observation of a high lung tumor incidence in the Schneeberg mines (Bohemia) is now generally correlated with the presence of radioactive substances;[401, 402]

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384. R. Biltris: Bull. Assoc. franç. p. l'étude du cancer *22*: 438 (1933).
385. N. Petrov & N. Krotkina: Ztschr. f. Krebsforsch. *38*: 249 (1933).
386. N. Dobrovolskaia-Zavadskaia: Compt. rend. Soc. de biol. *119*: 360 (1935).
387. O. Schürch & E. Uehlinger: Schweiz. med. Wchnschr. *64*: 664 (1934); Arch. klin. Chir. *183*: 23; 704 (1935); Ztschr. f. Krebsforsch. *45*: 240 (1937).
388. J. C. Mottram: Proc. Roy. Soc. Med. *29*: 15 (1935).
389. H. Hellmer: München. med. Wchnschr. *84*: 980 (1937).
390. J. M. Ross: J. Path. & Bact. *43*: 267 (1936).
391. G. Roussy, Ch. Oberling & M. Guérin: Bull. Acad. de méd. Paris *112*: 809 (1934); Bull. Assoc. franç. p. l'étude du cancer *25*: 716, (1936).
392. F. R. Selbie: Lancet *2*: 847 (1936).
393. G. Prussia: Sperimentale, Arch. di biol. *90*: 522 (1936).
394. S. Miyamoto: Strahlentherapie *64*: 683 (1939).
395. H. B. Andervont & M. B. Shimkin: J. Nat. Cancer Inst. *1*: 349 (1940).
396. L. Foulds: Am. J. Cancer *35*: 363 (1939).
397. K. Ullmann in Jadassohn: Handbuch der Haut-und Geschlechtskrankheiten, *12*: p. 531 (1933).
397a. H. S. Martland: Am. J. Cancer *15*: 2435 (1931).
398. E. Uehlinger: Schweiz. Ztschr. f. allg. Path. u. Bakt. *1*: 444 (1938).
399. G. Abruzzese: Riv. ital. ginec. *13*: 64 (1931); abstr. Ztschr. f. Krebsforsch. *36*: Ref. 101 (1932).
400. F. Norgaard: Am. J. Cancer *37*: 329 (1939).
401. A. Pirchan & H. Sikl: Am. J. Cancer *16*: 681 (1932).
402. H. R. Döhnert: Ztschr. f. Krebsforsch. *47*: 209 (1938).

this appears to be substantiated by measurements of radioactivity in the mines [403] as well as by animal experiments.[404] Exposure of mice to the inhalation of radioactive mineral dust has been shown considerably to increase the incidence of lung tumors in these animals.[404a]

In this connection, it may be stressed that the striking capacity of radioactive substances for not only curing cancer but also for producing malignant tumors is not an isolated instance in tumor pathology; this "ambivalence" seems to be a certain rule as it is similarly evident in the case of X-rays, of arsenic and of carcinogenic hydrocarbons The personal view of the authors favors an explanation assuming an involvement of the reticulo-endothelial system in this mechanism. The opposite effects may result, depending on the degree of the same stimulus, in proportion as it causes an activation (cancer cure) or paralyzation (cancer production) of the reticulo-endothelial system [405] (cf. paragraph on reticulo-endothelial system).

As stated above, no discussion of radium therapy, that extremely valuable and extensively used specialty in cancer pathology, will be attempted here. But experiments will be discussed which, in contrast to local radium applications, tried to achieve a therapeutic effect by means of injections of soluble radioactive substances. In this case a selective adsorption of radioactive substances by tumor tissue has been presupposed [406] although—as in the case of bismuth and lead —such an assumption cannot be considered as experimentally proven. As long ago as 1904 a method was reported in which, by local injections of radium-emanation, a sterile necrosis, growth inhibition and occasional cures of tumors were observed.[407] Local, and especially intravenous, emanation injections were extensively used by French authors.[406] They reported an alleviation of pain in advanced cancer patients and, also; regression of tumors and metastases. Similar favorable results were stated in a later study which also indicated a delayed tar cancer formation in rabbits treated by this method.[408] Histologic examination made it probable that this effect might be attributed to an activation of the reticuloendothelial system. Another explanation of the emanation action was based on the finding that radium-emanation addition stimulated the autolysis of malignant tumors to a stronger degree, than it enhanced the autolysis of normal organs.[409] Preliminary animal experiments were performed to determine the toxicity of radium emanation, with the idea of using it clinically in diffuse pelvic carcinosis.[410] Mice bearing spontaneous and inoculated tumors were exposed to thorium emanation developed in closed cages; after sufficiently high dosage, necrosis and sometimes regression of the tumors was observed. The author stressed the preliminary character of these experiments.[411] Work with tissue cultures showed a growth inhibition of mouse fibroblasts and cancer cells by radium chloride and also by radium emanation. This effect was reversible, that is, after replantation of the cultures into radium-free media, growth was resumed.[412] This agrees well with a finding of an analogous reversibility of a respiratory inhibition of leucocytes caused by emanation.[413] These findings do not lend themselves to the encouragement of injection therapy of radioactive substances; the rapid disintegration of radium emanation, limiting the time of its effectiveness, together with the fact that a selective localization of these substances in the tumor seems uncertain, explain the failure of these therapeutic attempts to convince the clinician of their usefulness. Correspondingly, a review of these experiments caused the forming of negative conclusions.[414]

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[403] B. Rajewski: Ztschr. f. Krebsforsch. *49*: 315 (1939).

[404] W. Hueck: Ztschr. f. Krebsforsch. *49*: 312 (1939).

[404a] J. A. Campbell: Brit. M. J. *1*: 217 (1942).

[405] K. Stern: New Internat. Clin. Vol. III., Ser. *4*: 111 (1941).

[406] A. Kotzareff & R. Fischer: Les cancers et la physico-chimie, Paris, Vigot frères, 1927.

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[407] A. Braunstein: Therap. d. Gegenw. *45*: 412 (1904).

[408] F. Vanýsek: Ztschr. f. Krebsforsch. *38*: 94 (1932).

[409] S. Löwenthal & E. Edelstein: Biochem. Ztschr. *14*: 484 (1908).

[410] I. I. Kaplan: Am. J. Roentgenol. *37*: 675 (1937).

[411] H. Lederer: Helvet. med. Acta *6*: 395 (1939).

[412] T. Gordonoff & F. Ludwig: Schweiz. med. Wchnschr. *68*: 45 (1938).

[413] W. Fleischmann & D. Laszlo: Klin. Wchnschr. *16*: 1248 (1937).

On the other hand, it is highly inadvisable to administer radioactive substances of long lasting action into the body because of their incontrollable effect on normal tissues. Animal experimentation of this kind has been performed with varying results. Tumor takes in mice and rats were inhibited in 50 per cent of the animals by means of thorium X,[415] and the Ehrlich mouse carcinoma was retarded, or cured, by injections of thorium nitrate.[416] Experiments using thorium B, however, gave completely negative results, both as to its localization in mouse tumors and also as to any therapeutic effect.[417, 418] The use of thorium dioxide (thorotrast) which was employed, combined with roentgen radiation, both in animal experiments [419, 420] and in cancer patients [421] with moderately good results must be discarded because of the high carcinogenic property of this substance.[391-396] Even the use of thorium dioxide for diagnostic purposes (mammography) cannot be considered harmless; after observation of subsequently arising conditions suspected of being cancer, caution was urged in using this procedure.[422]

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[414] J. G. Hamilton & R. S. Stone: Radiology *28*: 178 (1937).

[415] M. Yamauchi: Ztschr. f. Krebsforsch. *21*: 230 (1924).

[416] M. Montanari-Reggiani: Arch. ital. sc. farmacol. *2*: 201 (1933); abstr. Ztschr. f. Krebsforsch. *40*: Ref. 57 (1933).

[417] R. Ehrenberg: Ztschr. f. Krebsforsch. *35*: 348 (1933).

[418] C. Dittmar: Ztschr. f. Krebsforsch. *48*: 121 (1938).

[419] Ch. Oberling & M. Guérin: Bull. Assoc. franç. p. l'étude du cancer *22*: 469 (1933).

[420] S. Russ & G. M. Scott: Proc. Roy. Soc. London, s. B *118*: 316 (1935).

[421] H. Bosch: München. med. Wchnschr. *80*: 907 (1933); 1st Internat. Cancer Congress, Madrid II: 477 (1933).

No beneficial effect on tumors was seen from actinium administration.[423] Ionium was reported even to enhance the tar carcinoma formation in rabbits.[147] The primary source of radioactive substances, uranium, was also employed clinically. The authors,[424, 425] although occasionally achieving regression of metastases by means of intravenous uranium-thorium injections, never observed a permanent influence on the tumor or even a prolongation of the life of the patients treated. The intratumoral injections of a uranium preparation into tumors of the female sex organs caused necrosis and decomposition, but as a general application this treatment cannot be recommended because of the unavoidable severe complications (perforations, general intoxication).[426]

8. *Theory of Metal Action.*

Although manifold phenomena appear to be involved in the influence exerted on tumor development and growth by the elements discussed, in those cases of animal experimentation where favorable results were obtained, it was suggestive to look for a common denominator of their mechanism. The fact that a far reaching resemblance of the metal actions is recognizable according to their form of application, and partly independent of their chemical nature, seems to favor the conception that an unspecific mechanism is at least partly involved. Among the various application forms, the repeatedly mentioned use of colloidal metal sols and complex organic metal compounds is to be recalled. A procedure of more recent date is iontophoresis. By this method beneficial results in animal experiments were seen from lead, silver, copper and barium salts [427, 428] and also in a few clinical cases by the employing of zinc and copper [429] or magnesium [430] for iontophoresis. A facilitated permeation of the chemical into the tissue is attributed to iontophoresis, but this procedure is limited to use in superficial tumors only. Furthermore, it was stated [428] that the necroses occurring in tar cancer of mice, following calcium or lead iontophoresis, only rarely result in permanent cures and they are frequently followed by recurrences. Therefore, the clinical use of iontophoresis does not seem promising even in skin cancer.

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[422] C. D. Collins: Canad. M. A. J. *40*: 440 (1939).

[423] V. Czerny & A. Caan: München. med. Wchnschr. *58*: 1801 (1911).

[424] F. D. M. Hocking: Internat. Conf. Cancer, London 1928, 253.

[425] G. T. Pack & F. W. Stewart: J. Cancer Res. *14*: 152 (1930).

[426] H. Kamniker: Strahlentherapie *40*: 427 (1931).

There are principally three theories which attempt to establish a common denominator for the action of metals on tumors. The first was proposed by Neuberg and Caspari.[258] On the basis of extensive animal experimentation, these authors considered the common cause of the chemotherapeutic effect achieved by various metal compounds as being an increase of autolytic processes in the tumor. This autolysis is preceded by a hyperemia, and in favorably responding tumors liquefaction results followed by tumor disappearance. The authors noted an essentially uniform behavior of the tumors following the intravenous injection of most widely different metals. They employed organic complex compounds for the purpose of increasing the tumor affinity and by this means they investigated the action of tin, lead, arsenic, antimony, vanadium, mercury, cobalt, copper, gold, platinum, silver, iridium, ruthenium, osmium, palladium, rhodium on mouse carcinomas, rat sarcomas, and on a spontaneous dog cancer.

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427. A. Borrel, A. de Còulon & L. Boez: Compt. rend. Soc. de biol. *87*: 1118 (1922).
428. N. Waterman: Ergebn. d. inn. Med. u. Kinderh. *30*: 304 (1926).
429. Durham 1919, quot. Waterman, l. c. 428, p. 334.
430. R. Reding & A. Slosse, abstr. 428, p. 334.

Copper, tin and, particularly, silver and cobalt proved to be highly effective; the tumor hyperemia appeared a few minutes after the first injection, hemorrhages occurred, and a few days later the tumor was generally completely liquefied. Histologic analyses of the tumor during this stage of the treatment revealed the presence of the injected chemicals in the tumor, or in its detritus. The authors assumed a colloidal nature for these metal deposits, increasing their effectiveness. The chemical analysis of the liquefied tumor confirmed the supposition of an increased autolysis since almost no unchanged proteins but, instead, abundant low-molecular incoagulable protein decomposition products were found. In favorably reacting instances continued treatment resulted in resorption of the cystic tumor or in perforation to the outside and, subsequently, in cure without recurrence of the tumor. The authors, however, pointed out the essential difficulty, that therapeutic doses were very close to lethal ones. This was especially disturbing, since in using too small doses tumor stimulation was even observed. It is scarcely necessary to mention that the explanation for the action of the metals by their autolysis-increasing property may be valid for other of the experiments mentioned above. Further, it is to be stressed that the metal activity is obviously not merely dependent on the colloidal state but, also, that the complex compounds used by Neuberg & Caspari exhibit additional suitability for chemo-therapeutic purposes. In this connection attention is drawn to investigations in which intravenous injection of various metal sols (copper, silver, mercury, antimony) failed to exert a therapeutic effect on mouse carcinoma.[220] Similarly, a curative ineffectiveness (spontaneous mouse carcinomas) was reported of fifty simple inorganic compounds.[481] A transfer of the results of Neuberg & Cas-

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431. M. C. Marsh: Am. J. Cancer *22*: 572 (1934).

pari to human pathology did not succeed and in this connection it must be considered that, together with the high toxicity of the metal compounds, the rapid resorption of the large amounts of highly toxic tumor decomposition products formed would also present a grave danger for the life of the patient.

A second explanatory attempt [432] was based on the fact that following intravenous injection of various chemotherapeutically effective substances, thromboses and other damages of the tumor-supplying vessels were noted. Hence, the already a priori insufficient blood supply of the neoplasms was assumed to be additionally impaired, causing necroses and regressive changes. But in this connection it must be pointed out that the administration of thrombogenic agents was not found to inhibit the growth of animal tumors, even if used together with lead compounds.[377]

A third theory [433] placed the chemotherapeutic action not into the tumor itself, but envisaged a stimulation of the defense mechanism of the diseased organism. The reticuloendothelial function was especially claimed to be activated by the stimulus of metal localization (cf. section on reticuloendothelial system).

B. NON-METALS.

1. *Halogens.*

Chlorine. Analytical data of the chlorine content of tumors are scarce. An increase to four times the concentration of surrounding muscle was found in Rous sarcoma. In the blood serum and other organs, excepting the muscle, the chlorine showed a tendency to decrease.[434] A chlorine in-

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432. C. Lewin: Ztschr. f. Krebsforsch. *27*: 132 (1928).
433. W. Caspari: Internat. Conf. Cancer, London 1928, 199.
434. V. Morávek: Ztschr. f. Krebsforsch. *37*: 293 (1932).

crease, however, was reported to be present in the blood of Brown-Pearce carcinoma rabbits,[9] and of rats inoculated with Ehrlich-Putnoky carcinoma.[435] In disagreement with an older statement of a chlorine increase,[436] later studies did not reveal changes of the chlorine-level of the blood of non-cachectic tumor patients.[437, 11] In severe cachexia only, a hypochloremia was noted,[438, 11] which was especially marked in patients suffering from gastric cancer.[439] The diminution or absence of free hydrochloric acid in the gastric contents of these patients is commonly known. But it was also claimed that a similar finding may be present in the gastric juice of patients affected with tumors of other localization.[439, 440] Further studies and explanations of this phenomenon are contained in the chapter on physical chemistry.

As to the chlorine metabolism, a general tendency to retention seems to prevail in malignant tumors,[441-443] in which in addition to the neoplasm the liver is mainly involved.[444] This chlorine retention was increased by a NaCl-rich diet in rats bearing spontaneous and transplanted tumors.[445, 435] This behavior was correlated to hepato-renal disturbances,[435] is not constantly found in cancer patients,[446] and is also present

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435. S. Sümegi: Frankfurt. Ztschr. f. Path. *48*: 386 (1935).
436. W. von Moraczewski: Virchow's Arch. f. path. Anat. *139*: 285 (1895).
437. M. Mairano: Minerva med. (Torino) 1931, II: 820; abstr. Ztschr. f. Krebsforsch. *36*: Ref. 78 (1932).
438. F. Martinez Nevot & M. Gallego Burin: Arch. españ. oncol. *3*: 33 (1932); abstr. Ztschr. f. Krebsforsch. *38*: Ref. 53 (1932).
439. B. Moore, W. Alexander, R. E. Kelly & H. E. Roaf: Biochem. J. *1*: 274 (1906).
440. F. W. M. Palmer: Biochem. J. *1*: 398 (1906).
441. R. Laudenheimer: Ztschr. f. klin. Med. *21*: 513 (1892).
442. G. Setti: Riv. veneta di Sci. med. *30*: 113 (1899); abstr. Maly's Tierchemie *29*: 741 (1899).
443. H. H. Glosser & W. S. Frisbie: J. A. M. A. *46*: 561 (1906).
444. A. Robin: Compt. rend. Acad. d. sc. *156*: 1409 (1913).
445. K. A. Winter: Ztschr. f. Krebsforsch. *40*: 410 (1934).
446. A. Schöpp: Deutsche med. Wchnschr. 1893: 1155; 1213.

in inflammatory conditions.[447] The spontaneous chlorine excretion in the urine of cancer patients showed uncharacteristic changes, and supernormal values were occasionally observed.[448] As to therapeutic use, it was recommended that the radiosensitivity of tumors be increased by means of NaCl-augmentation [447] without defining whether the anion or the cation is the active factor.

Iodine. Few reliable data are available as to the iodine content of neoplasms because recent methods, only, warranted precise iodine determination of biological material. However, a generally valid rule was claimed according to which the iodine content and, especially, the soluble protein iodine increase with the vitality of tissues; this was reported to be the case in non-necrotic cancer tissue also.[449] As a matter of fact, in mouse and rat tumors the iodine concentration was found to exceed that of liver and muscle.[450] The finding that a bone tumor, histologically proven as metastasis of a thyroid carcinoma, exhibited approximately the same iodine content as the thyroid [451] is of interest inasmuch as it affirms the functional relation between tumor and metastasis. The blood iodine level of cachectic tumor patients was determined to be mostly subnormal; but as supernormal values also were not too rarely found in cancer patients, no characteristic behavior is apparent in this respect.[452] The organs of tumor-bearing mice and rats showed higher iodine concentrations than those of control animals, excepting the thyroid, in which a diminished iodine content was noted.[450]

The iodine deposition in tumors is a debated question.

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447. E. Andersen: München. med. Wchnschr. *71*: 933 (1924).
448. A. Braunstein: Ztschr. f. Krebsforsch. *1*: 199 (1904).
449. A. Sturm & L. Rockmann: Biochem. Ztschr. *287*: 50 (1936).
450. H. Toyoda, S. Kishi & W. Nakahara: Gann *29*: 29 (1935).
451. E. Gierke: Hofmeister's Beitr. *3*: 286 (1903).
452. F. S. Fowweather: Brit. J. exper. Path. *11*: 400 (1930).

Whereas some authors[453, 454, 450] alleged a high iodine storing ability of tumors, another investigator[455] grouped cancer between the iodine-poor and the iodine-rich tissues following iodine administration. The specific nature of the iodine localization in tumors was further restricted by the observation that an increased iodine deposition occurred only after onset of regressive changes in the tumor.[456] Later analyses showed that following injection of iodide the concentration of this element in animal tumors equaled that of normal organs.[457] Following roentgen radiation the iodine localization of tumors was found to be reduced.[304]

In spite of this doubtful theoretical basis iodine was used for tumor therapy. Presupposing its tumor affinity, it was used together with cerium ("Introcid").[458, 221] In a reexamination, this treatment caused growth retardation of tumors and general improvement, but these effects were attributed only to an unspecific action of introcid, causing protein decomposition.[459] Combined application of potassium iodide and radiation therapy has been claimed to yield beneficial clinical results.[460, 461] Moderate growth inhibition and increased radiosensitivity was also seen in the Kato rabbit sarcoma, following injections of potassium iodide and of introcid.[462] Potassium iodide, however, proved to be ineffective in transplanted mouse carcinoma, if used alone or in combination with radiation therapy.[463] Probably no specific iodine

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453. R. von den Velden: Biochem. Ztschr. *9*: 54 (1908).
454. F. Jess: München, med. Wchnschr. *68*: 323 (1921).
455. M. Takemura: Ztschr. f. physiol. Chem. *72*: 78 (1911).
456. G. Holler & F. Singer: Biochem. Ztschr. *139*: 199 (1923).
457. H. Ito: Jap. J. Obst. & Gynec. *21*: *267* (1938).
458. C. Lewin: Med. Klin. *20*: 1317 (1924).
459. B. Cohn: Deutsche med. Wchnschr. *51*: 1984 (1925).
460. I. Bagge: Ztschr. f. Krebsforsch. *10*: 251 (1911).
461. G. Riehl: München. med. Wchnschr. *68*: 644 (1921).
462. H. Ito: Jap. J. Obst. & Gynec. *20*: 536; 541; 545 (1937).
463. W. Moppett: Med. J. Australia *1*: 785; *2*: 15 (1936).

action underlies experiments in which iodized glycogen caused regression of rat tumors in 50 per cent of the treated animals.[464] This result, however, is in contrast to the tumor promoting effect frequently observed of unchanged glycogen (cf. organic chemistry). Especial significance must be attributed to exactly controlled animal experiments, in which no basis for a cancer therapeutic effect of iodine was revealed. Although small iodine amounts inhibited growth of mouse and rat tumors, this could be related to the impairment of the general health of the animals only.[465]

Finally, it may be stated that attempts to use tincture of iodine as a carcinogen failed. Prolonged administration to the skin of mice resulted in inflammation and hyperkeratosis, but never in atypical epithelial growth.[466]

Fluorine. In spite of earlier negative results [467] a renewed attempt was made to utilize fluorine for tumor therapy [468, 469] because of the antiglycolytic property of this substance.[470, 471] Peroral application of fluorides failed to influence rat tumors; intratumoral injection caused extensive necrosis. Addition of sodium fluoride, though inhibiting growth of cancer tissue cultures and impairing the transplantability of mouse tumors,[472] did not affect inoculated growing tumors.[473] Therapeutic experiments with fluorides cannot be seriously considered for clinical use on account of their high toxicity. An organic, less toxic compound, 3-fluortyrosine, was used recently in animal experiments. Peroral or subcutaneous administration inhibited development and growth of Jensen rat sarcoma, Ehrlich mouse carcinoma and benzpyrene tumors of rats and mice.[474] No effect of fluortyrosine, however, was noted in experiments on ascites carcinoma of mice. Even admixture of the fluorine compound to the tumor material did not impede its transplantability.[475]

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[464] A. Borrel & A. de Coulon: Compt. rend. Soc. de biol. *86*: 1096 (1921).

[465] K. Sugiura & S. R. Benedict: Am. J. Cancer *23*: 541 (1935).

[466] J. Rosenstirn: J. Cancer Res. *10*: 61 (1926).

[467] S. Fränkel & E. Fürer: Wien. klin. Wchnschr. *29*: 198 (1916).

[468] W. Ewig: Klin. Wchnschr. *8*: 759 (1929).

[469] B. Fischer-Wasels: Die Gasbehandlung bösartiger Geschwülste, Frankfurt a. M. 1930, p. 91.

[470] W. Ewig: Klin. Wchnschr. *8*: 839 (1929).

[471] C. Sellei & J. Jány: Biochem. Ztschr. *239*: 94 (1931).

[472] B. Lustig & E. Werber: Ztschr. f. Krebsforsch. *43*: 364 (1936).

[473] J. C. Krantz, Jr., R. Musser, C. J. Carr & W. G. Harne: Am. J. Cancer *30*: 332 (1937).

2. *Nitrogen Group (Phosphorus, Arsenic, Antimony).*

Phosphorus. As an introduction, it must be stated that the analytical data reported frequently do not refer to the inorganic phosphorus alone, but also to that obtained after ignition of the substance thus including organic phosphorus, i.e., phosphorus occurring in phospholipids and nucleic acids. This form of phosphorus is evidently increased in cell-rich tissues. On the other hand, a relationship may undoubtedly be expected to exist between the inorganic phosphorus and the high phosphatase activity inherent in tumor tissues.[476] In certain stages of tumor development, the increased phosphatase splitting must lead also to an increased liberation of phosphorus from the tumors. Apart from an older statement, and therefore from the point of view of methods not fully valid,[1] most of the analyses indicated a reduced phosphate content of tumors.[477] In particular, a diminution of the orthophosphates was noted.[478] The total acid-soluble phosphorus was determined in mouse carcinomas as being 90–100 mg. per cent, in mouse sarcomas as 70-75 mg. per cent, while 170–180 mg. per cent were found in the muscle of mice.

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[474] H. May & G. Litzka: Ztschr. f. Krebsforsch. *48*: 376 (1939).

[475] K. Niedner: Ztschr. f. Krebsforsch. *51*: 159 (1941).

[476] S. Edlbacher & W. Kutscher: Ztschr. f. physiol. Chem. *199*: 200 (1931).

[477] D. P. Barr, E. Ronzoni & J. Glaser: J. Biol. Chem. *80*: 331 (1928).

[478] K. W. Buchwald: J. Cancer Res. *14*: 536 (1930).

Similarly, other phosphorus fractions were also determined as being reduced.[479] Analogous findings were obtained in the Rous fowl sarcoma,[480] and in these animals an increase of the total blood phosphorus corresponded to the reduced phosphorus content of the neoplasm.[481] A marked rise of the inorganic phosphorus was also established for the blood serum of Brown-Pearce carcinoma rabbits.[482] The blood plasma of cancer patients, however, showed only an insignificantly elevated inorganic [10] and acid-soluble [483] phosphorus. It remains obscure, at present, because of the unreliable methods employed and the lack of a more recent check-up, how justified a claim was, made long ago, which inferred that tumor-free bones of cancer patients possess an increased phosphorus content.[484]

Especial interest has been devoted to the phosphorus content of erythrocytes. The ratio $P_2 0_5$: red blood cells ("phosphorus-ratio") was found to be raised in cancer patients and diagnostic significance was attributed to this phenomenon.[485] Re-investigation, although confirming this finding, could not prove it as specific,[486, 487] as similar changes of the phosphorus ratio were also apparent in other conditions, e.g., in icterus. Continued work asserted the nonspecificity of this phenomenon,[488, 489] as depending on anemia [489] or on cachexia.[490] Experiments on the Rous sarcoma showed that this changed phosphorus ratio was clearly only a secondary consequence of the tumor process since the increase of the phosphorus ratio reached its maximum only in the second or third week after the tumor inoculation and exhibited oscillations not correlated with the tumor growth.[491]

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[479] A. Goldfeder: Acta Cancrol. *2*: 533 (1936).

[480] V. Morávek: Ztschr. f. Krebsforsch. *37*: 365 (1932).

[481] F. Pentimalli & G. Schmidt: Biochem. Ztschr. *282*: 62 (1935).

[482] A. R. Harnes: J. Exper. Med. *50*: 109 (1929).

[483] A. Goldfeder: Ztschr. f. Krebsforsch. *40*: 394 (1934).

[484] Beneke; Deutsches Arch. f. klin. Med. *15*: 538 (1875).

[485] W. Gröbly: Arch. f. klin. Chir. *115*: 170; 261 (1921).

[486] Joh. Vorschütz & Jos. Vorschütz: Deutsche med. Wchnschr. *48*: 861 (1922).

[487] H. Zerner: Ztschr. f. Krebsforsch. *21*: 157 (1924).

[488] T. E. Buckmann, G. R. Minot, G. A. Daland & M. Weld: Arch. Int. Med. *34*: 181 (1924).

[489] F. B. Byrom & H. D. Kay: Brit. J. Exper. Path. *8*: 429 (1927); *9*: 72 (1928).

[490] F. Bernhard: Mitt. a. d. Grenzgeb. d. Med. u. Chir. *38*: 204 (1924).

Another series of studies was devoted to the relative phosphorus distribution between red blood cells on the one side, and the serum or plasma, on the other. The respective ratio

$$\frac{\text{Total phosphorus in red blood cells}}{\text{Total phosphorus in plasma}}$$

was given as an average of five for normal persons.[492] While cancer-free patients exhibited similar values, cancer patients were claimed to yield much higher ratios (average 7.5, maximum 11.5).[493] Following roentgen radiation of cancer patients, a reduction of the heightened ratio to normal values was reported.[494] Hopes of utilizing this behavior for diagnostic purposes, however, were destroyed by a check-up comprising more than 1000 cases, as this study did not reveal a regular deviation of the phosphorus distribution between red blood cells and the plasma of cancer patients.[495]

Interesting light was thrown on the subject of phosphorus metabolism of malignant tumors by investigations employing the radioactive ("labeled") phosphorus isotope. By this means the phosphorus uptake of animal tumors (mammary carcinoma, lymphosarcoma, lymphoma of mice) was studied and compared with normal soft tissues. The tumors showed

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491. E. Haam & R. Stöhr: Klin. Wchnschr. *8*: 2382 (1929); Biochem. Ztschr. *220*: 399 (1930).

492. G. E. Youngburg & M. V. Youngburg: J. Lab. & Clin. Med. *16*: 253 (1930).

493. H. L. Brose & E. B. Jones: Med. J. Australia *23*: 506 (1936).

494. H. L. Brose & E. B. Jones: Nature, London *138*: 687 (1936).

495. J. B. Thiersch & S. Schlosser: Med. J. Australia *28*: 77 (1941).

a phosphorus uptake during the early intervals equal to the uptake of the most active normal tissues (liver, kidney) but were distinguished from them by the capacity for retaining the "labeled" phosphorus for long periods.[496] This was confirmed not only for animal tumors [497, 498] but also for human neoplasms (especially lymphosarcoma and osteogenic sarcoma). [499, 500] The significance of these findings is heightened by recent reports which indicate the possibility of successfully using the radio-active phosphorus isotope (P^{32}) for the treatment of certain malignant tumors, especially lymphosarcomas.[500, 501] Additional experiences with this therapy may be expected in the near future and thus render possible a more precise evaluation of this new form of cancer treatment.

Arsenic. No analytical data are available as to the arsenic content of malignant tumors. The assumption—at present completely abandoned—that the arsenic content of tar is a factor of its carcinogenicity led to arsenic analyses of organs and tumors of tarred animals and no deviation from the norm was detected.[502] The arsenic storing ability of the tumor is far inferior to that of normal tissues, especially of liver and of skin.[503]

Substantial significance must be attributed to arsenic as a carcinogenic factor. In this connection the experiments of Carrel [504] and of Askanazy,[505] respectively, seemed most im-

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496. H. B. Jones, I. L. Chaikoff & J. H. Lawrence: Am. J. Cancer *40*: 235; 243 (1940).

497. A. Marshak: Science *92*: 460 (1940).

498. L. W. Tuttle, L. A. Erf & J. H. Lawrence: J. Clin. Investigation *20*: 57 (1941).

499. J. M. Kenney: Surgery *9*: 327 (1941).

500. J. M. Kenney, L. D. Marinelli & H. Q. Woodard: Radiology *37*: 683 (1941).

501. J. M. Kenney: Cancer Research *2*: 130 (1942).

502. A. H. Roffo & M. L. Correa: Bol. inst. de méd. exper. para el estud. y trat d. cáncer *2*: 367 (1926).

503. A. S. Minot: J. Cancer Res. *10*: 293 (1926).

504. A. Carrel: Compt. rend. Soc. de biol. *93*: 1083 (1925).

505. M. Askanazy: Wien. klin. Wchnschr. *40*: 609 (1927).

pressive. These authors succeeded in producing malignant tumors (fowl sarcomas) by implantation of embryonic tissue to which arsenic had been added, though embryonic tissue without arsenic failed to result in tumor production. It was also reported that in vitro normal monocytes were transformed into malignant cells by the addition of arsenic acid [506] and the inoculation of fowl sarcoma together with minimal arsenic amounts increased the malignancy of the ensuing tumors.[507] The general validity of these observations, however, has been doubted, since some authors [508-511] were unable to reproduce the previously mentioned experiments of Carrel and Askanazy, while others [512, 513] confirmed their findings. The complex nature of this phenomenon is apparent from a study according to which the sarcoma formation after injection of arsenic containing embryonic tissue was feasible only in animals fed a vitamin B-rich diet.[514]

Another series of experiments has dealt with the direct application of arsenic to the skin. By the application of Fowler's solution to mice, epitheliomas were produced in two to three per cent of the animals and metastasis formation occurred in one instance,[515] while prolonged administration of a ten per cent arsenic ointment provoked a cancroid in one out of ten rabbits.[516] Experiments with the feeding of arsenic,

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506. E. Haagen: Deutsche med. Wchnschr. *54*: 92 (1928).
507. L. Califano: Riv. pat. sperim. *6*: 113 (1930); abstr. Ztschr. f. Krebsforsch. *34*: Ref. 212 (1931).
508. W. A. Collier & I. Hartnack: Ztschr. f. Krebsforsch. *30*: 131 (1929).
509. F. Kauffmann: Ztschr. f. Krebsforsch. *30*: 136 (1929).
510. G. Felloni: Pathologica *22*: 256 (1930).
511. N. C. Laclau & C. Pillado-Matheu: Compt. rend. Soc. de biol. *103*: 1285 (1930).
512. A. W. M. White: J. Cancer Res. *11*: 111 (1927).
513. N. Petrov & N. Krotkina: Bull. Assoc. franç. p. l'étude du cancer *18*: 688 (1929).
514. V. Bisceglie: Riv. pat. sperim. *8*: 364 (1932).
515. A. Leitch & E. L. Kennaway: Brit. M. J. *2*: 1107 (1922).
516. L. S. Raposo: Compt. rend. Soc. de biol. *98*: 86 (1928).

however, yielded negative results,[502] for which possibly the too short life duration of the animals was responsible. Actually, in a later study, production of lung tumors in two out of twenty mice was reported after feeding arsenic for one year (daily 0.02–0.04 mg. As_2O_3), and a sarcoma of the ear arose in a rabbit after arsenic feeding for half a year.[517]

A vast literature deals with the possibility of a carcinogenic activity of arsenic in human beings.[518–520] Two methods must be considered: external and internal application. The first possibility refers mainly to persons professionally dealing with material containing arsenic, such as leather workers using arsenic acid as a preserving substance for hides and furs. In England, where arsenic acid is employed for wool cleaning, skin cancer is frequently observed in persons handling this arsenic powder.[397] The mysterious lung cancer, occurring in some mines (Schneeberg, Bohemia), was formerly connected with the presence of arsenic,[204] but recent studies point decisively to radio-active substances as being responsible for these tumors.[401–404] Likewise, work of recent years has revealed the invalidity of earlier assumptions claiming a connection between arsenic admixtures and the carcinogenic property of soot, tar and aniline derivatives (cf. organic chemistry).

The causal connection between internal arsenic administration and cancer formation appears better founded. As an aftermath of arsenic medication, prolonged for many years as was usual, e.g., in psoriasis, in addition to arsenic melanosis and hyperkeratosis, development of epitheliomas was also noted. Analogous observations were made following uncontrolled prolonged arsenic application for other reasons[397, 516, 517] and, on the basis of these facts, the prolonged arsenic administration must be watched carefully, especially during the age disposed to cancer. An interesting explanation for the carcinogenicity of arsenic could be seen in findings according to which this substance selectively damages dehydrogenases, i.e., an important partial enzyme of respiration, thus impairing the oxidation metabolism.[521, 522]

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[517] J. Cholewa: Ztschr. f. Krebsforsch. *41*: 497 (1935).

[518] C. C. Franseen & G. W. Taylor: Am. J. Cancer *22*: 287 (1934).

[519] S. Fassrainer: Zentralbl. f. Chir. *63*: 25 (1936).

[520] F. Voss: Strahlentherapie *66*: 155 (1939).

Likewise, in arsenic—analogous to radio-active elements—an ambivalence of a cancer producing property and, at the same time, of a cancer curing property has been observed. For empirical reasons, internal arsenic medication has been employed in malignant tumors for a long time. Such a treatment was even claimed to accomplish the complete cure of skin cancer if treatment started not later than half a year after the onset of the disease.[523] An experimental basis is furnished by a study demonstrating an inhibition of tar cancer of mice by intraperitoneal injections of As_2O_3.[524] Similar results were obtained in tar cancer of mice by peroral $As_2 O_3$ administration.[525] Numerous, at present abandoned, therapeutic experiments, employing organic arsenic compounds (Atoxyl, Salvarsan)[526] will be mentioned in the chapter on organic chemistry. The sometimes successful use of caustic arsenic pastes (Zeller's paste) in skin cancer goes back many years. Prolonged application of one to two per cent arsenous solutions were also recommended as an effective treatment for skin cancer.[527] A nostrum ("Abjitin-ointment"), containing arsenic as effective constituent, provoked discussion re-

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521. G. Barry, E. Bunbury & E. L. Kennaway: Biochem. J. *22*: 1102 (1928).
522. A. Szent-Györgyi: Biochem. J. *24*: 1723 (1930).
523. O. Lassar: Ztschr. f. Krebsforsch. *3*: 516 (1905).
524. W. Schiller: Ztschr. f. Krebsforsch. *23*: 39 (1926).
525. E. Puccinelli: Pathologica *22*: 63 (1930).
526. F. Blumenthal: Berl. klin. Wchnschr. *50*: 1942; 1993 (1913).
527. Trunczek: Berl. klin. Wchnschr. *37*: 741 (1900).

cently,[528, 529] but it scarcely can be expected that this or other arsenic pastes will obtain a permanent place in the treatment of skin cancer in view of available, much more effective and reliable radiation therapy.

Antimony. Animal experiments showed that the injection of tartar emetic prevented the takes of inoculated tumors if applied half an hour preceding the graft.[530] A destructive effect on rat carcinoma was ascribed to antimony salts of fatty acids.[302] It was also used clinically: the employed preparation "Antiblastoma" contained in addition to tartar emetic an ipecac extract, thus combining two highly active emetics. This treatment was claimed to have resulted in thirty-nine cures and fifty-eight improvements out of 155 cancer cases treated during a period of two years, and the best results were seen in adenocarcinomas.[531] In contrast to this report no beneficial influence from this treatment on the cancerous process was observed in Fichera's institute.[532] Further experimentation with transplanted animal tumors also indicated only a small effectiveness of antimony salts of fatty acids [428] and of antimony tartrate, respectively.[533]

3. *Oxygen Group (Oxygen, Sulfur, Selenium, Tellurium).*

Oxygen. In view of the unique, vital importance of this element for the metabolism, therapeutic attempts will be discussed in connection with respiration.

Sulfur. It is customary in biochemistry to devote separate consideration to sulfate sulfur, to ether sulfur and to neutral sulfur. Strictly, the discussion here should be con-

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[528] B. Aschner: Die Krise in der Medizin, Hippokrates-Verl. Leipzig 1928.

[529] K. Funke & G. Scherber: Wien. klin. Wchnschr. *46*: 1563 (1933).

[530] P. Salmon: Compt. rend. Soc. de biol. *86*: 200 (1922).

[531] R. Minervini: Internat. Cancer Congress Madrid I: 462 (1933).

[532] P. Pattarin: Giorn. med. Alto Adige 1930; abstr. Fichera l.c. 173.

[533] S. Shibuya & S. Inaba: Tr. Jap. Path. Soc. *23*: 691 (1933).

fined to the sulfate sulfur, but in the interest of clear arrangement the other categories, i.e., organically bound sulfur, will also be referred to in part, especially since many investigations have dealt with total sulfur. A reduction of the sulfur content was found in human liver cancer [1] and also in Rous sarcoma.[534] It was assumed that this decrease depended mainly on a reduction of protein sulfur. A detailed account of this problem comprising sulfur-containing amino acids (cysteine, glutathione), will be given in the chapter on organic chemistry, in the paragraph on proteins.

The sulfur content of other organs of fowls affected with Rous sarcoma was reported to be unchanged.[534] The sulfur concentration of mouse and rat tumors was found to be lower than that of the kidney and the brain, but higher than that of muscle, blood, heart and liver; generally the tissues of tumor-bearing animals were richer in sulfur than those of normal animals.[450] These varying and ambiguous results may be explained by a communication, according to which the total sulfur content of the skin increases proportionally to the growth rate; this was claimed also to hold good for mouse carcinoma.[535] An observation of a peculiar nature referred to changes of the sulfur content of the hair in patients afflicted with neoplasia; in male persons suffering from cancer, a decrease of the sulfur was claimed to occur in the hair, and this was especially marked in intestinal cancer, whereas in female cancer patients only slight changes were noted, and the presence of sarcomatous tumors was accompanied in both sexes by an increase of the sulfur in the hair.[536] It is obvious that these findings would indicate an alteration of the chemical composition of the hair keratin, viz. an organic compound, but at present the cause and the significance of

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534. V. Morávek: Ztschr. f. Krebsforsch. *37*: 299 (1932).
535. H. Brown & J. V. Klauder: J. Lab. & Clin. Med. *20*: 1143 (1935).
536. K. S. Kosyakov: Bull. Biol. et méd. exper. U.R.S.S. *57*: 407 (1939).

this phenomenon remain obscure and are in need of further confirmation.

Several papers deal with the excretion of neutral sulfur in the urine of cancer patients. The abundant presence of a neutral, easily to SO_4 oxidisable sulfur fraction was found in these cases.[537, 538] An identity of this substance with thiocyanate was made probable and a diagnostic utilization of this finding was proposed.[538] Although these results were essentially confirmed,[539–541] no specific nature was attributed to them. One will scarcely go amiss in viewing this phenomenon merely as an expression of an increased protein decomposition.[542] This is made the more probable as other high molecular protein decomposition products containing sulfur (oxyproteic acids) excreted in the urine of cancer patients were also the subject of extensive investigations (cf. organic chemistry).

Although unwarranted by the scarce analytical data mentioned, a supposed sulfur deficiency was alleged to be a causal factor of cancer formation [543–545] and, correspondingly, parenteral sulfur administration was recommended for cancer therapy. Intravenous injection of colloidal sulfur preparations were reported to cause growth inhibition, regression, and even the cure of inoculated mouse and rat tumors.[546, 547] The clinical use of this treatment has been claimed as resulting in similar beneficial results. Re-examinations, however,

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537. H. Salomon & P. Saxl: Wien. klin. Wchnschr. *24*: 449 (1911).
538. P. Saxl: Biochem. Ztschr. *51*: 224 (1913).
539. N. Murachi: Biochem. Ztschr. *41*: 138 (1912).
540. M. Mazzitelli: Riforma med. *28*: 813 (1912).
541. H. Kahn: Klin. Wchnschr. *1*: 4 (1925).
542. A. Friedrich & E. Bauer: Ztschr. f. physiol. Chem. *242*: 261 (1936).
543. Grumme: Ztschr. f. Krebsforsch. *33*: 367 (1930).
544. G. Stern: Klin. Wchnschr. *9*: 37 (1930).
545. A. Tanaka: Tr. Soc. jap. path. *24*: 531 (1934).
546. G. Izar & C. Basile: Tumori *2*: 65 (1913).
547. L. Zagni: Tumori *13*: 42 (1927).

did not consistently corroborate these claims, inasmuch as favorable sounding reports [548, 549] are contrasted by negative ones; Fichera [173] saw no effect from the sulfur treatment. The mechanism was explained as a stimulation of tumor autolysis and the analogy to the supposed therapeutic action of metal colloids is obvious. Another theory, however, explained a growth retardation of rat tumors observed following sulfur injections by a changed electrical charge of the tumor cells.[550] Other therapeutic experiments were concerned with the use of intravenous sodium thiosulfate injections,[551] and with external application of concentrated potassium thiocyanate solutions.[552] Both measures were used in skin cancers and favorable results were reported. But the insufficient number of experiences collected and the better response of most skin cancers to therapeutic measures does not permit the drawing of general conclusions from these reports.

Selenium and *tellurium.* The use of these substances represented a landmark in experimental cancer research. From the historic standpoint, one is, therefore, justified in dealing more extensively with this subject than would correspond with its present significance. In 1911 A. Wassermann [553] announced to the scientific world that he had succeeded in accomplishing remarkable therapeutic effects in mouse tumors by intravenous injections of an eosin-selenium compound. He and his collaborators had observed, incidentally, that tumor cells exhibited, in vitro, a high affinity for selenium and tellurium salts. The suggestive idea of utilizing this fact for therapeutic purposes was found to be justified, insofar as

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[548] Piccinini, Zagni & Volta; Roubin & Bournigault: quot. Fichera, 173.

[549] O. Cignozzi: Riforma med. *43*: 682 (1927).

[550] D. C. A. Butts: Cancer *1*: 234 (1924).

[551] V. Kutka: Bratislav. lék. listy *10*: 883 (1931).

[552] A. Tarabuchin: Arch. f. Dermat. u. Syph. *168*: 519 (1933).

[553] A. v. Wassermann, F. Keysser & M. Wassermann: Deutsche med. Wchnschr. *37*: 2389 (1911).

local injection of sodium tellurite or sodium selenite actually caused necroses of mouse tumors. At this point credit must be given to Wassermann for the basic conception that such a local action is insufficient in practical cancer therapy for two reasons: One, because it would be clinically usable in a limited number of cases only; two, because its success even in these cases would presuppose that the disease was not generalized. Correspondingly, the author was obliged to attempt the induction of the tumor destroying substance into all diseased foci by the humoral way, i.e., by intravenous injection. Such experiments performed on animals, however, failed entirely, obviously because the chemicals mentioned did not reach the tumor in sufficient concentration. In view of this situation Wassermann found it necessary to combine the cancer destroying agents with another substance which, though by itself ineffective, would serve, so to speak, as pathfinder by virtue of its tumor affinity. For this purpose he tested some dyes of high diffusibility. After protracted experiments he believed he had found the desired preparation in a selenium eosin compound. Intravenous injections of this substance were administered to tumor-bearing mice. For the necessary prolonged treatment, a special technic of injection was worked out, permitting up to eight injections into the tail vein. Following the third injection, a liquefaction of the tumor occurred and, finally, the tumor was transformed into a fluctuating cyst, the contents of which were resorbed. A cure, not followed by recurrence, was achieved within an average of ten days presupposing that the tumor had not reached a too large size. In this latter case, however, animals died due to the resorption of too large amounts of toxic cell disintegration products. The treatment was found to be equally effective in transplanted carcinoma, sarcoma and spontaneous tumors of mice. Independently of this work, but at the same time, similar results were obtained by intravenous injections of an 0.1 per cent sodium tellurite solution.[554] Here, too, after liquefaction, tumors were resorbed and the animals did not succumb to recurrences. It remains obscure as to why this author was able to bring about a sufficiently high tellurium concentration in the tumors without using a vehicle. Regarding the theoretical explanation of the selenium and tellurium action, it was reported that these salts inhibited the autolysis of normal tissues, but activated this process in neoplasms.[555] It is obvious that these relations exhibit a striking analogy to the results and to the theoretical basis of the studies of Neuberg & Caspari [258] in which complex organic metal compounds were employed. The great expectations for human cancer therapy which were connected with these apparent successes of animal experiments met, however, with complete disappointment. In view of the large number of publications, which were mostly confined to reports on a few cancer cases treated with various selenium preparations, it may suffice here to refer to the summary given by Fichera.[178] On the basis of the literature [556-561] and of his own experiences (fourteen cases), he arrived at the conclusion that the beneficial effects of the selenium therapy in animal experiments—these, too, were not generally confirmed [556]—were not paralleled as far as human pathology was concerned. Although isolated reports of older [562] and of newer date [563] claimed certain favorable clinical results, an exact clinical investigation of col-

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554. L. Grillo: Policlinico (sez. prat.) *19*: 1185 (1912).
555. G. M. Fasiani: Arch. soc. med. *36*: 436 (1912).
556. Ch. E. Walker: abstr. Wien. klin. Wchnschr. *25*: 1462 (1912).
557. A. Jeremics: Berl. klin. Wchnschr. *51*: 381 (1914).
558. O. Pankow: abstr. Berl. klin. Wchnschr. *53*: 1327 (1916).
559. Touche: Bull. Soc. méd. Paris *35*: 451 (1913).
560. E. Watson-Williams: Brit. med. J. *2*: 463 (1919).
561. A. Turletti: Therapia *22*: 268 (1932); abstr. Am. J. Cancer *22*: 152 (1934).
562. A. Braunstein: Berl. klin. Wchnschr. *50*: 1102 (1913).
563. A. T. Todd: Brit. J. Surg. *21*: 619 (1934).

loidal selenium therapy, comprising seventy cancer cases, led to such discouraging results[564] that it was completely abandoned. Finally, recent experiments employing intravenous injections of diselenide diacetic acid, and diselenide n-dibutyric acid, failed to cause any growth inhibition of the mouse sarcoma 180; neither has an increased concentration of selenium been observed in the tumors of the treated animals.[565]

4. *Carbon Group (Carbon, Silicon, Titanium, Germanium).*

The material on *carbon* is dealt with in the chapter on organic chemistry.

Silicon. But little light has been thrown on the significance of this element for tumor pathology. As to the neoplasm itself, only one old and, in method, unreliable analysis indicated an increased silicon content of human liver carcinoma.[1] Reports of an increased silicon content of the pancreas of tumor patients[566, 567] were not confirmed by a later investigation.[568] The alleged diminished silicon excretion in the urine of cancer patients[566] could, even if definitely established, be considered of value only if the decisive nutritional factors were to be exactly controlled.

Several studies have been devoted to the statistical analysis of a correlation between silicosis and lung cancer.[569-572] It cannot yet be conclusively decided as to whether the high pulmonary cancer rate in persons affected with silicosis is due

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564. Medical Committee of the Royal Cancer Hospital: Lancet *1*: 1198 (1936).

565. S. B. Gusberg, P. Zamecnik & J. C. Aub: J. Pharmacol. & Exper. Ther. *71*: 239 (1941).

566. H. Kahle: München. med. Wchnschr. *61*: 752 (1914).

567. H. Schulz: Biochem. Ztschr. *70*: 464 (1915).

568. W. Kasten: Frankfurt. Ztschr. f. Path. *50*: 42 (1936).

569. D. H. Dible: Lancet *2*: 982 (1934).

570. M. J. Fine & J. V. Jaso: J.A.M.A. *104*: 40 (1935).

571. K. M. Lynch & W. A. Smith: Am. J. Cancer *24*: 56 (1935).

572. O. Klotz & W. Simpson: Contr. to the Med. Sciences, Internat. Press, New York, 1932, Vol. 2, p. 685.

to a specific chemical action, or whether it is merely caused by an unspecific mechanic irritation. On the basis of an extensive statistical analysis, the first possibility was cautiously advocated.[573] In animal experiments (mice) exposure to silica or iron dust significantly increased the incidence of lung tumors.[574] As to a therapeutic use of silicates in human cancer, only very few cases were reported without the giving of histologic data.[575]

Titanium. A tumor growth inhibiting effect was attributed to titanium hydroxide and its salts, on the basis of a few animal experiments only. Complete tumor regression was not observed.[576, 577]

Germanium. In a very uncritical way a "cancer curing" quality of a natural mineral spa in Germany (Dunaris fountain, Eifel) was alleged by lay persons, and the presence of germanium in this water was at the same time connected with this supposed curative action.[578] No experimental material is available as to the influence of germanium on tumors.

C. *Water*

The discussion of inorganic tumor chemistry would be incomplete without dealing with the role of water in tumor pathology. It is true that this cell constituent has a double significance insofar as it is not only involved in the chemical, but also preeminently in the physico-chemical cell structure. This second factor will be discussed in detail in the chapter on physical chemistry.

Even a long time ago an increased water content of malignant tumors had been ascertained[579] and has since frequently been confirmed.[580-582] This behavior was to be expected, inasmuch as all young, growing tissues, i.e., especially, embryonic tissue, have a high water content. Nevertheless, the supposition held by some[583] of a direct ratio between water content and malignancy, i.e., growth energy of tumors, has not been generally recognized.[581] In particular, the significant finding has been reported that in a liver affected with cancer not only the tumor but also the apparently healthy liver tissue showed an increased water content as compared with a normal liver.[579] This is the more striking since in tuberculosis of the liver the diseased parts showed a higher hydration, but in the surrounding tissue the water content was even diminished. The author assumed a connection between hydration and vital activity of the cells, because he found the fatty infiltrated liver cells of alcoholics to be exceedingly poor in water.

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[573] M. O. Klotz: Am. J. Cancer *35*: 38 (1939).

[574] J. A. Campbell: Brit. M. J. *2*: 275 (1940).

[575] A. Zeller: München. med. Wchnschr. *54*: 1841; 1916 (1912).

[576] S. von Pastinszky & B. Ottenstein: Ztschr. f. Krebsforsch. *42*: 245 (1935).

[577] D. Abragam: Compt. rend. Acad. d. Sc. *200*: 990 (1935).

[578] A. Neumann: Wien. med. Wchnschr. *81*: 1077 (1931).

Analogous results have been obtained in animal experiments. Rat carcinomas and sarcomas were analyzed, and the tumors exhibited the highest water content (75 per cent). But the blood and metastases-free liver of tumor-bearing animals also showed a water concentration two to three per cent higher than the respective organs of normal animals.[582] An increased water content of the whole body was observed in tumor-bearing mice: seventy-five per cent as compared with 66.5 per cent in normal animals.[584] Similar results were obtained in investigations of mouse carcinoma and rat sarcoma; the former contained eighty to eighty-three per cent water, the latter showed one to two per cent lower values, while normal muscle was found to contain sixty-eight to seventy per cent water,[585] and in sarcoma-bearing fowls an increased hydration and, correspondingly, a reduced ash content was demonstrated in tumor, lung and blood.[586] The water content of the livers of rats bearing large carcinosarcoma 256 was significantly higher than in the liver of control animals, and this increase was not influenced by diet, and could not be accounted for by a decrease of the dry residue.[587]

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[579] A. Robin: Bull. Acad. de méd. Paris *81*: 699 (1913).

[580] A. H. Roffo & J. Thomas: La chímie du cancer, Paris, Vigot frères, 1933.

[581] W. Marvelli: Tumori *16*: 212 (1930).

[582] H. Schlottmann & W. Rubenow: Ztschr. f. Krebsforsch. *36*: 120 (1932).

[583] R. Simonini: Clin. pediatr. *6*: 1 (1924).

[584] A. Bolaffi: Tumori *15*: 18 (1929).

These phenomena could be attributed to the following factors: One, the presence of hydration-promoting ions potassium, sodium, chloride; two, the presence of similarly effective protein decomposition products and, three, a change of the lipid composition, influencing the cell permeability. Especial emphasis was laid upon the last factor, as a parallel was claimed to exist between water and cholesterol content.[580] It was noted that in mice the ratio (water content of tumor)/(water content of body) approximately paralleled the ratio (cholesterol in tumor/cholesterol in body). This correlation between cholesterol and water content appears supported by comparing the analyses of the non-necrotic periphery and of the necrotic center of rat carcinosarcoma 256. Here too, the central part which was richer in cholesterol and cholesterol esters showed a higher water content than the cholesterol-poorer periphery.[588] It must, however, be mentioned that another author [589] viewed the disturbed water metabolism of cancer patients as a secondary phenomenon only, depending on liver damage and nephrotic changes. As to the possibility

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585. A. Goldfeder: Acta Cancrol. *2*: 333 (1936).
586. V. Morávek: Ztschr. f. Krebsforsch. *35*: 492 (1932).
587. H. D. McEwen & F. L. Haven: Cancer Research *1*: 148 (1941).
588. F. L. Haven: Am. J. Cancer *28*: 57 (1937).
589. J. Putnoky & S. Sümegi: Ztschr. f. Krebsforsch. *41*: 505 (1935).

mentioned of inorganic ions influencing the hydration, attention is drawn to a study, according to which a potassium-rich diet increased the water content of tissues of normal mice.[590] Hence the discussed potassium increase in tumors could also be responsible for the augmented hydration and, likewise, the reduced virulence of tumor material, achieved by treatment with calcium chloride solution, has been explained by a dehydration and protoplasma condensation due to the calcium action [591] (cf. also physical chemistry, discussion of "free" and "bound" water in tumors [592]).

An insight into the water metabolism of the body may be gained by the McClure & Aldrich test, which consists of producing a wheal by intracutaneous saline injection and of the measuring of the time necessary for the disappearance of the wheal. This test was performed in fifty-seven cancer patients, and showed a delayed liquid resorption, but this behavior was interpreted as expressing only the impaired general condition of the patients.[593]

From an experimental point of view, the influence of severe thirsting—water deprivation—was tested on tumor-bearing rats.[594] A special affinity of water for the tumor was observed, because its water content, in contrast to the other tissues, did not show an appreciable reduction as compared with control animals. A therapeutic effect, however, was not noted in this study, nor in subsequent analogous experiments in which the administration of diuretics was also ineffective.[595] On the other hand, a very high sensitivity of cancer tissue cultures against water privation was reported; malignant tissue succumbed much earlier than even embryonic tissue of the same water content. This agrees well with the finding that desiccation of tumor tissue, e.g., over calcium chloride, destroyed its transplantability.[580] The "infectious" fowl sarcomas, however, must be excepted in this respect and the characteristic behavior of these tumors is discussed in another place. A connection with therapeutic measures is given by a paper stating that a decreased water content of neoplasms follows effective irradiation.[583] In experiments on the mouse sarcoma 180 an increased radiosensitivity was observed after injection of distilled water, while injections of hypertonic or isotonic Ringer solution were ineffective.[596] This phenomenon could be correlated with changes of the physico-chemical structure, but its use for radio-therapeutic purposes must await continued experimentation.

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[590] Gerard, abstr. H. Roffo & J. Thomas, 1. c. 580.

[591] W. Cramer: Rep. Imp. Cancer Res. Fund. *7*: 1 (1921).

[592] N. Okuneff & T. Nasarbekowa: Ztschr. f. Krebsforsch. *41*: 28 (1934).

[593] V. Podetti: Riv. chir. *2*: 493 (1936); abstr. Ztschr. f. Krebsforsch. *45*: Ref. 304 (1937).

[594] R. Guastalla: Cancro *2*: 98 (1931); abstr. Ztschr. f. Krebsforsch. *35*: Ref. 29 (1932).

[595] M. J. Shear: Am. J. Cancer *25*: 66 (1935).

"Heavy" water (deuterium oxide) was also introduced into experimental cancer research, and the deuterium oxide content of animal tumors was not found to differ from normal organs. In some investigations injections of this substance failed to influence mouse [597, 598] or rat tumors [599] but, while in these experiments the achieved deuterium oxide concentration in the organism was comparatively low, another study employed a saturation method, i.e., by prolonged peroral administration the deuterium oxide concentration in the animals was brought to about thirty per cent. Mice treated in this way after inoculation of highly virulent carcinoma or sarcoma showed a much lower growth rate (tumor weight one half or less, than in controls). A decision could not be made

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596. K. Sugiura: Am. J. Roentgenol. *43*: 533 (1940).
597. W. H. Woglom & C. A. Weber: J.A.M.A. *102*: 1289 (1934).
598. K. Sugiura & L. C. Chesley: Proc. Soc. Exper. Biol. & Med. *31*: 659 (1934).
599. C. E. Rea & S. Yuster: Proc. Soc. Exper. Biol. & Med. *31*: 1058 (1934).

as to whether this effect depended on a specific action (enzyme inhibition?) or was merely the expression of a generally toxic reaction affecting the nutrition of the animals.[600]

Summary.

It would be understandable if the material presented in this chapter. were to evoke a completely negative attitude toward the work accomplished thus far and cause a pessimistic evaluation of continued studies in this field. But such an attitude appears, in the authors' opinion, to be by no means justified. With regard to the available material, on the one hand, it must not be forgotten that in cancer research, as well as in science generally, negative results—frequently obtained with no less investment of labor and talent than positive ones—are of decisive importance insofar as they narrow by exclusion the range of problems to be considered. On the other hand, a number of positive achievements mentioned must not be neglected or underrated. And, epecially, in this respect, the possibilities appear by no means exhausted for, on the contrary, the basis seems to be laid for continued investigations, as will briefly be shown.

Although a direct causal connection between tumor development and inorganic factors cannot be accepted, some secondary changes accompanying the tumor process have been made very probable (potassium, calcium, zinc, etc.). By means of extensive clinical analysis attempts may be made to put these findings on a broader basis, and to utilize them for purposes of classification, for evaluation of therapeutic response of tumors, and for general prognostic viewpoints. Furthermore, it is known that the course of diseases may be decisively influenced by inorganic factors not etiologically responsible for the respective pathology. The same may hold good for tumor pathology, and studies should be undertaken of a much broader scope and in a more exact way than has been the case up to now. In this respect a careful re-examination is desirable with reference to the selective or increased localization in malignant tissues which has been attributed to some inorganic substances. Although the present evidence is not conclusive in any instance in regard to such a phenomenon, the possibility of its existence cannot as yet be precluded. It is obvious that positive findings of this nature could open new therapeutic pathways.

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[600] **H. G. Barbour & E. Allen: Am. J. Cancer *32*: 440 (1938).**

Insofar as chemotherapeutic attempts are concerned, it must be postulated that old and new recommendations of this kind should first be tested by reliable animal experimentation. Emphasis should be put on a suitable form of application (parenteral or peroral) while intratumoral administration scarcely seems able to furnish conclusive results. Although it cannot be expected that in human cancer pathology the use of inorganic substances alone will yield a generally effective therapy, it seems to be within the range of possibility that such measures might support radiation therapy (increased radiosensitivity) and that selected tumor cases might be beneficially influenced.

A direct practical significance must be attributed to the detection of carcinogenic qualities for certain inorganic substances, as by this means occupationally caused neoplasms may be recognized and the way for their prevention be prepared. Experimental analysis should accompany and enhance these studies, especially, because the solution of a carcinogenic mechanism in each single case can be of theoretical importance, contributing to our knowledge of the cancer problem.

These concluding suggestions should, of course, not be misinterpreted in a manner inferring that work based upon them must necessarily meet with success, or that no other ways could be proposed which might also lead to valuable results. As the most important basis for successful work in the sphere of inorganic tumor chemistry, there must be demanded an attitude focusing attention upon the tumor problem as a whole, and closely connecting theoretical deliberations and experimental studies with the results obtained in other fields of cancer research.

CHAPTER II

ORGANIC CHEMISTRY

A CONVENTIONAL viewpoint would hold it self-evident that the organic chemistry of a biological problem represents the most important and most explanatory part of its chemical elaboration. The fact that this natural assumption has not been established as a matter of course in the realm of cancer research must be reason for reflection. At least, as far as purely analytical data are concerned, proven findings of characteristic nature, distinguishing malignant tumors from the norm or from other pathologies, are but few, and even here it is still debatable whether, and to what extent, these data are connected primarily with the cancerous process, or instead represent only secondary changes. These restricting remarks refer mainly to the first section of this chapter dealing with the organic constituents of neoplasms. The second and the third sections are devoted to organic substances which, though foreign to the tumor and to the body, have been the subject of studies in relation to their tumor producing, or to their tumor curing properties, respectively.

A. *Organic Constituents of Tumors and Their Hosts.*

The following material is arranged in such a way as to discuss first, the native, complex constituents like polymer carbohydrates and proteins and, then, their simpler structural parts or decomposition products, such as monosaccharides, amino acids, etc. Touching on some facts belonging to the chapter on metabolism, or on enzymes cannot be avoided. Because the intake of vital organic material exerts an important influence on the tumor process, points of contact with the chapter on nutrition must naturally also take place, although the detailed discussion of vitamins is reserved entirely for that chapter.

1. *Carbohydrates.*

Glycogen. The majority of the pertinent investigations showed neoplasms to have a high glycogen content, exceeding that of the respective matrix. As long ago as 1885, an abundance of carbohydrates was demonstrated as being present in some malignant tumors.[1] For reasons mentioned later, it appears permissible to identify partly this carbohydrate content with glycogen. The high glycogen content of various human and animal tumors was histologically proven by several authors,[2] as far as quantitative conclusions may be derived by this method. As a good example, a study may be referred to which showed an abundance of glycogen in a gastric cancer, whereas the rest of the stomach contained only traces.[3] A direct correlation between glycogen content and the grade of malignancy of neoplasms has even been deduced from the results of extensive investigations.[4-6] A glycogen concentration of approximately 1% of the fresh weight was chemically determined in human tumors.[7] Comparisons with the matrix showed the tumor glycogen to be increased up to twenty to thirty times the normal amount.[8] An investigation of benign and of malignant tumors furnished, for the latter, values about ten times higher than for the former, while the glucose content did not differ appreciably,[9] and an elevated carbohydrate content was also observed in human lung cancer.[10] As demonstrated on the Ehrlich mouse carcinoma, the carbohydrates of the tumor consisted mainly of glycogen, whereas glucose presented an insignificant part only. Therefore one appears justified in correlating findings of the total carbohydrate content in tumors with glycogen, even if no specific methods have been used. The total carbohydrate content of mouse tumors was given as approximately 0.2%.[11, 12] Attention is called to an older report which stated that an increased glycogen level was also found in the tumor-free tissues of the tumor host.[13]

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[1] E. Freund: Verh. Kongr. f. inn. Med. 1889, p. 404.

[2] Schiele (1880); Cornil & Ranvier (1881); Lukjanow (1895); quot. A. H. Roffo & J. Thomas: La Chimie du Cancer, Paris, Vigot frères, 1933, p. 85.

[3] T. Saiki: Arch. de méd. exper. *33*: 376 (1911).

[4] A. Brault: Presse méd. *1*: 37 (1898).

[5] B. Sokoloff & C. Carlotto: Compt. rend. Soc. de biol. *89*: 628 (1923).

[6] O. Pentagna: Gazz. internat. di med. e chirurg. 1914, No. 27–28; abstr. Ztschr. f. Krebsforsch. *15*: 519 (1915).

[7] T. Langhans: Virchows Arch. f. path. Anat. *120*: 28 (1891).

As to the significance of these results, those tumors must be excepted, the high glycogen content of which depends on their being derived from a glycogen-rich parental tissue, such as chondromas, hypernephromas and hepatomas. In this connection a study of a transplantable mouse hepatoma, which showed the glycogen content of the tumor cells to be equal or slightly higher than that of normal liver cells, is of interest.[14] Furthermore, it must be borne in mind that embryonic tissue also contains large amounts of glycogen.[15] This circumstance suggests the interpretation of this behavior as a quality common to young, or rapidly growing tissue. In addition, glycogen augmentation is also connected with inflammatory conditions and with circulatory disturbances,[15] factors which are also present in neoplasms. The unspecific nature of glycogen deposits has been established in regard to the epidermis of adult humans, for this tissue is considered to be glycogen-free, with the exception of the hair roots and the sweat glands, but a marked glycogen presence was demonstrated in inflammatory epithelial granulations, in the surrounding of abscesses and in malignant tumors.[16] In addition, the glycogen increase is not shared by all malignant tumors, since it was reported that mammary cancers contain no glycogen, except in rare instances.[17-19] Traces of glycogen only were found in the Jensen rat sarcoma for which the high amylase activity, observed in the tumor, could possibly be responsible.[20]

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[8] G. Joannović: Wien. klin. Wchnschr. *39*: 345 (1916).

[9] F. Bernhard: Ztschr. f. klin. Med. *98*: 50 (1924); Klin. Wchnschr. *7*: 1184 (1928).

[10] B. Lustig: Klin. Wchnschr. *15*: No. 13 (1936); Biochem. Ztschr. *284*: 367 (1936).

[11] E. Wertheimer: Arch. f. d. ges. Physiol. *223*: 619 (1930).

[12] C. F. Cori & G. T. Cori: J. Biol. Chem. *64*: 11 (1925).

[13] B. Goldmann: Beitr. z. klin. Chir. *78*: 83 (1912).

[14] L. C. Strong & G. M. Smith: Bull. Assoc. franç. p. l'étude du cancer *26*: 694 (1937).

[15] E. v. Gierke, in L. Aschoff: Pathol. Anatomie, Fischer, Jena 1928, p. 423.

Some practical importance may be attributed to the interesting findings of Schiller;[21] this author noted that even in incipient stages cancers of the uterine cervix are glycogen-free in contrast to the normal matrix and this finding seemed constant enough to warrant its use for early diagnosis of uterine cancer. The procedure consists either in painting the portio in situ with an aqueous iodine solution, by which means the brown color of the normal mucous membrane may be

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16. I. Rothman & F. Schaaf in Jadassohn: Handbuch der Haut-und Geschlechtskrankheiten, Berlin, Springer, 1929, Vol. I, p. 271.

17. O. Lubarsch: Ergebn. d. allg. Path. u. path. Anat. *2*: 166 (1895).

18. E. v. Gierke: Ergebn. d. allg. Path. u. path. Anat. *11*: 891 (1907).

19. B. Sokoloff: Compt. rend. Soc. de biol. *89*: 628 (1923).

20. S. Edlbacher & W. Baumann: Ztschr. f. Krebsforsch. *47*: 191 (1938).

21. W. Schiller: Virchows Arch. f. path. Anat. *263*: No. 2 (1927); Arch. f. Gynäk. *133*: No. 2 (1928); Zentralbl. f. Gynäk. *51*: No. 30 (1928); Wien. klin. Wchnschr. *44*: No. 49 (1931); Lancet *230*: 1228 (1936); Monatschr. f. Krebsbekämpf. *2*: 7 (1934).

distinguished from the whitish one of cancerous tissue, or epithelial sections are obtained by biopsy and subjected to the staining and histologic analysis in vitro. The proof that glycogen was responsible for the brown staining of normal tissue was seen in the fact that a preceding treatment of the tissue with amylase resulted in the absence of the brown stain. As far as the practical value of the test is concerned, the author himself stressed that it is not absolutely specific, but that a positive result should arouse a suspicion of cancer.[22] Clinical experiences, using the test for such purposes, were generally favorable [23] although one study revealed the occurrence of some glycogen-rich cancers of the uterine cervix, therefore yielding misleading results with Schiller's test.[24] It is noteworthy that the glycogen in question is water-insoluble, in contrast to liver and muscle glycogen. Such a water-insoluble glycogen is also found in the epithelium of the larynx, in the epithelium of the esophagus and in early embryonic skin. Most of the enumerated glycogen analyses of tumors referred, however, to the typical water-soluble substance. It is of interest that chemical determinations also of water-soluble glycogen in uterine carcinomas showed a value of only 0.14 per cent of the fresh tissue weight.[25] Significantly, it was possible to differentiate the various forms of uterine cancer by means of their glycogen content: undifferentiated carcinomas of the cervix contained little or no glycogen, while this substance increased proportionately to the tumor differentiation.[26, 27] In this connection, reference is made to the observation that Walker rat tumors 256, the growth rate of which had been slowed down by hypophysectomy of the hosts, contained much larger concentrations of glycogen than the more rapidly growing tumors of intact animals.[28] Closer analysis of this phenomenon made it very likely that the greater glycogen content of slow-growing tumors was due to a decreased utilization and a consequent accumulation of the carbohydrate.[29] Regarding chemically induced tumors, histologic examination of hepatomas, produced in rats by feeding butter yellow, revealed a complete absence of glycogen in the tumor cells.[30]

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[22] W. Schiller: New England J. Med. *218*: 878 (1938).

[23] Ch. E. Galloway: Am. J. Surg. *26*: 281 (1939).

[24] A. Babes: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *14*: 201 (1937).

[25] G. Tesauro: Arch. di ostet. e ginecol. *13*: 385 (1926); abstr. Cancer Rev. *2*: 219 (1926).

[26] W. Lahm: Ztschr. f. Geburtsh. u. Gynäk. *93*: 356 (1928).

[27] A. Babes & S. Pantzu-Lazarescu: Virchows Arch f. path. Anat. *275*: 765 (1930).

Summarizing, the glycogen content of malignant tumors appears to be influenced mainly by the following factors: one, by their origin, insofar as frequently a glycogen-rich parental tissue imparts this quality to the tumor arising from it; two, by the growth rate, inasmuch as it is accompanied by a greater or lesser utilization of carbohydrates; and three, by the grade of differentiation of tumors, i.e., the glycogen content tends to increase in proportion to the cell differentiation. The interdependence of these factors makes it understandable that variations of the glycogen content were found in different tumors according to their stage of development, localization, etc.

As to experimental results, addition of glycogen, obtained from human tumors, to mouse tumor transplants increased the yield of tumors.[31, 32] A particular affinity of tumor tissue for carbohydrates appears to have been established by several studies. In vitro carcinoma cells were reported as exhibiting a selective adsorption of glycogen, whereas sarcoma cells failed to do so.[33] Mouse tumors seemed to possess a great power for retaining carbohydrates in vivo, also, since various measures such as strychnine, lack of oxygen, low temperature decreased the sugar content of the whole body, but they did not affect the carbohydrate concentration in the tumor.[11] In vitro, glycogen protected cancer cells against the cytolytic action of normal serum (cf. section on carcinolytic test); in vivo, however, glycogen somewhat inhibited the growth of animal tumors.[34] However, we must consider that paravascular sugar application is liable to cause extensive tissue damage and, hence, impede tumor growth in a wholly unspecific manner. An extensive study was concerned with the polysaccharide of plant origin, viz. *starch,* in regard to its effect on tumors. Sterile starch granules were injected into transplanted mouse carcinomas and sarcomas simultaneously with the inoculation or after the tumor development and were also added to tissue explants. In vivo, the takes of the tumors were prevented or the developed tumors, respectively, were destroyed following such a procedure, whereas tissue cultures showed impaired growth. The mechanism of this action was recognized as depending on the accumulation of granulocytes provoked by the starch granules and on the consecutive tumor-destroying action of the granulocytes. Aqueous sugar solutions as well as other colloidal suspensions (carbon, carmine, manganese dioxide, melanin granules) produced neither a leucocytic response nor tumor destruction.[35]

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[28] H. F. Schott, L. T. Samuels & H. A. Ball: Proc. Soc. Exper. Biol. & Med. *37*: 410 (1937).

[29] H. A. Ball, H. F. Schott & L. T. Samuels: Cancer Research *2*: 146 (1942).

[30] J. E. Edwards & J. White: J. Nat. Cancer Inst. *2*: 157 (1941).

[31] R. Odier: Rev. med. Suisse 1910; quot. loc. cit. 2.
(1922).

[32] A. Borrel & A. de Coulon: Compt. rend. Soc. de biol. *86*: 1096

As to the origin of the tumor glycogen, two possibilities must be considered;[25] either an infiltration from the outside, or an intracellular formation. In support of the latter conception, a study may be mentioned, according to which the supplying artery of the Rous sarcoma exhibited a higher blood sugar level than the vein collecting the tumor blood.[36] Analogous findings were reported for human tumors.[37] Theoretical considerations and experimental results caused one author to assume a glyconeogenesis in the tumor, i.e., formation of the polysaccharide from non-sugar substances.[38] The glycogen content of animal tumors following the intravenous injection of various monosaccharides has been determined; it was reported that the pentoses, arabinose and, particularly, xylose caused a marked glycogen increase in the tumors, whereas glucose was only slightly effective.[39] This claim, however, must be accepted with reservation, since in general physiology a transformation of pentoses into glycogen has not been established.

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[33] E. Freund & G. Kaminer: Biochem. Ztschr. *46*: 470 (1912).

[34] B. Lustig & H. Wachtel: Ztschr. f. Krebsforsch. *41*: 468 (1935).

[35] R. Chambers & C. G. Grand: Am. J. Cancer *29*: 110 (1937); *36*: 369 (1939).

Dextrins, decomposition products of polysaccharides, have been dealt with only inasmuch as they were found to inhibit tumor growth in a manner similar to glycogen, although they did not affect carcinolysis in vitro.[34]

In connection with carbohydrate metabolism, there will be occasion to refer to the important process of phosphorylation, a phenomenon extensively studied, which has been found of great importance for carbohydrate utilization in muscle. Here it need only be stated that the presence of hexose-phosphoric esters has been established in human and animal tumors.[40]

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[36] K. Tadenuma, S. Hotta & J. Homma: Biochem. Ztschr. *137*: 536 (1923).

[37] P. Mauriac, L. Servantie & Rioux: Compt. rend. Soc. de biol. *108*: 971 (1931).

[38] A. Brault: Ronas Ber. *61*: 655 (1931); Bull. Assoc. franç. p. l'étude du cancer *27*: 208 (1938).

[39] B. Borghi: Boll. soc. ital. biol. sper. *2*: 6 (1927).

[40] E. L. Outhouse: Trans. roy. soc. Canada IIIs, *27*: 123 (1933).

Glucose. This monosaccharide, so important in biochemistry, also received special attention in tumor chemistry. As mentioned above, the neoplasms themselves contain but very small amounts of glucose.[11] This is not surprising since this sugar represents the most easily disposable form of carbohydrate, destined as direct source of energy, and larger glucose reserves are not deposited in tissues. It is the carbohydrate used for transportation to the places of consumption and, therefore, is found mainly in the humoral media. Correspondingly, numerous studies have been devoted to the blood sugar level of cancer patients and animals. A hyperglycemia was found more or less regularly in clinical cancer cases.[41–46] Untreated patients, as well as a great many treated cancer patients, were reported to exhibit blood sugar values 20 per cent higher than the norm while fasting.[47] The elevated blood sugar level of patients suffering from uterine cancer was observed to revert to the norm, following radical surgery or successful radiation therapy.[48] So far as animal experiments are concerned, a hyperglycemia was found in fowl bearing Rous sarcoma,[44] but conflicting claims were presented regarding rat tumors. No characteristic changes of the blood sugar values were noted in rats inoculated with Flexner-Jobling carcinoma.[49, 50] Jensen sarcoma rats even

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[41] F. Silberstein, J. Freud & T. Revész: Ztschr. f. d. ges. exper. Med. *55*: 78 (1927).

[42] E. Schneider & H. Achelis: Klin. Wchnschr. *7*: 1955 (1928).

[43] R. Reding: Le Terrain Cancereux et Cancérisable, Paris, Masson 1932.

[44] H. M. Dyer & J. H. Roe: Am. J. Cancer *18*: 888 (1933).

[45] D. G. Krizschin, Z. M. Erusalimski & S. I. Aisenberg: Med. Z. vseukrain Akad. nauk *4*: 1153 (1935); abstr. Ztschr. f. Krebsforsch. *44*: Ref. 68 (1936).

[46] E. Aujaleu, F. H. Colombies & A. Montariol: Compt. rend. Soc. de biol. *120*: 954 (1935).

[47] G. E. Woodward & E. G. Fry: Biochem. J. *26*: 889 (1932).

[48] R. Bolaffi: Riv. ital. di ginec. *14*: 37 (1932).

[49] Y. Ihara: Mitt. allg. Path. u. path. Anat. *7*: 103 (1932).

[50] T. Fuke: Jap. J. Obst. & Gynec. *16*: 353 (1933).

showed a lowered blood sugar level and, at the same time, a decreased glycogen content in the liver.[51] Other authors, however, reported that rats inoculated with Jensen sarcoma exhibited blood sugar values rising in proportion to the tumor advancement, and that a decline of the glycemia became manifest only a few days before the death of the animals.[52] These findings, together with clinical experiences indicating an oscillating behavior of the blood sugar level of cancer patients,[53] suggest that the glucose concentration of the blood undergoes variations depending on the stage of the tumor process and on the nutritional status of the patients.

Special attention has been given to the so-called proteid sugar in the blood of cancer patients, i.e., to that part of blood carbohydrates which acquire reducing power after hydrolysis of the proteins only and which are determined in this way. An increase of this fraction in the blood of cancer patients had been reported long ago,[54] and this has since been confirmed by several authors.[55, 56] An increase of the proteid sugar to four times the normal value was claimed to occur in the venous plasma of cancer patients [57] and it was stressed that such an increase was encountered in carcinoma but not in sarcoma patients.[58] Animal experiments yielded analogous results regarding the Ehrlich carcinoma of mice and the Brown-Pearce carcinoma of rabbits. In this study, the protein precipitates were isolated, freed of other reducing substances, and then were subjected to hydrolysis and to the reduction test, thus insuring more accurate results. Repeated determinations showed that the proteid sugar increase parallels the tumor development.[59] But all the publications concerned, and especially more recent work,[60] emphasized that this change was not specific for malignant disease, but could be found likewise in tuberculosis, in chronic suppuration, and in liver damage. This relation with processes involving protein decomposition was also suggested by an investigation of the glucosamine-part of the proteid sugar in the blood of patients suffering from various diseases. An increase was observed in advanced malignancy only, whereas initial stages exhibited normal values. Since similar findings were obtained in infections (pneumonia) and in sterile infarcts,[61] it seems probable that tissue destruction, whether caused by malignant tumors or by any other process, may result in liberation and influx into the blood of proteins, distinguished from the native blood proteins by a higher carbohydrate content. Naturally, such an explanation divests the findings mentioned of any specific significance in regard to malignancy.

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[51] B. Purjesz & S. Lajos: Ztschr. f. Krebsforsch. *43*: 280 (1936).

[52] M. W. Lepskaja & A. F. Goldberg: Ztschr. f. Krebsforsch. *44*: 432 (1936).

[53] V. Jura: Boll. acad. med. Roma *59*: 195 (1933).

[54] E. Freund: Wien. med. Blätter No. 9, p. 36 (1885).

[55] N. Trinkler: Zentralbl. d. med. Wiss. p. 485 (1890).

[56] A. Grevenstuk: Ergebn. d. Physiol. *28*: 1 (1929).

[57] H. Bierry, F. Rathery & Levina: Compt. rend. Acad. d. sc. *173*: 56 (1921).

[58] B. Lustig & A. Langer: Biochem. Ztschr. *242*: 320 (1931).

Another sugar-like blood fraction was determined by means of its capacity for reducing chromic acid. Measurements of this kind, expressed as "chromic index," were supposed to denote the "residual sugar," after deduction of the glucose value found in the same specimen. The respective values were found increased in about eighty per cent of the cancer cases, although this finding, too, was not declared to be specific for malignant tumors.[62] Following radiation therapy of tumors, a reduction of this sugar fraction was

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59. F. Lasch & B. Lustig: Ztschr. f. Krebsforsch. *43*: 146 (1936).
60. K. Hinsberg & R. Merten: Ztschr. f. klin. Med. *135*: 76 (1938).
61. R. West & D. H. Clarke: J. Clin. Investigation *17*: 173 (1938).
62. C. Grandclaude, M. Polonovski, J. Driessens & H. Warembourg: Compt. rend. Soc. de biol. *111*: 22 (1932).

noted.[63] This "residual sugar" is only partly identical with the proteid sugar, since it obviously also contains other reducing substances such as sulfhydryl compounds.

Normal glucose values have been obtained by analysis of the spinal fluids of cancer patients.[64]

Significance of glucose in the tumor process. The carbohydrates, and particularly glucose, which represent the most important source of energy for every kind of cell, are also of paramount importance in the development and growth of malignant tumors, as has been proven by a great number of studies. As far as the intensive promotion of tumor growth by carbohydrate-rich diet [65] is concerned, a detailed account will be given in the chapter on nutrition. Intravenous and peroral glucose administration were seen to enhance the growth of a rabbit sarcoma [66] and of a rat carcinoma,[67] and extensive investigation demonstrated an acceleration of the growth of Flexner-Jobling rat carcinomas by means of glucose injections. The life of the treated animals was markedly shortened, and histologic analysis indicated a high-grade malignancy of their tumors.[49] Furthermore, glucose injections were found to promote atypical epithelial growth in mice treated with tar, and to enhance the growth of inoculated mouse tumors [68] and, in consequence of an augmented carbohydrate supply, animal tumors showed a higher concentration of both free and proteid sugar.[69] Some conflicting reports denied an influence of glucose and of glycogen on mouse

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63. C. Grandclaude, M. Polonovski, H. Warembourg & J. Driessens: Bull. Assoc. franç. p. l'étude du cancer, *25*: 58 (1936).

64. A. Mashbitz & Mazkevitsch: Zentralbl. f. Gynäk. *59*: 577 (1935).

65. M. Haendel: Ztschr. f. Krebsforsch. *21*: 288 (1924).

66. T. Kato: Tr. Jap. Path. Soc. *13*: 194 (1923).

67. J. Homma & T. Isshiki: Tr. Jap. Path. Soc. *17*: 537 (1927).

68. P. Rondoni: Biochim. e terap. sper. 1926, 1; Klin. Wchnschr. *5*: 465 (1926).

69. B. Kellner & B. Lustig: Biochem. Ztschr. *254*: 214 (1932).

tumor growth,[70, 71] but more recent work even indicated a direct carcinogenic action of glucose and other sugars. In the course of studies on tumor production in rats by the feeding of o-aminoazotoluene (hepatoma formation), subcutaneous glucose injections were administered, in order to increase the carcinogenesis. Contrary to expectations, a sarcoma formation at the site of the glucose injection was observed, and further experiments resulted in the formation of such tumors by means of glucose injections alone, without feeding of aminoazotoluene.[72] This sarcoma production in rats by means of daily subcutaneous glucose injections, which were continued for one year, has been confirmed;[73, 74] galactose and fructose were also found to exert the same effect.[74] It was suggested that the failure of another worker to reproduce these results was based on the genetic difference of the animal strain employed.[75]

On the other hand, a therapeutic use of intravenous glucose injections was proposed in combination with radiation therapy of tumors. An increased radiosensitivity of the neoplasm and an improvement of the general condition of the patient were claimed as resulting from such treatment.[76] Confirmatory results were reported on the basis of animal experiments,[77-79] and of clinical experiences,[80-82, 82a] but negative

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[70] S. Cioffari & N. Piccaluga: Tumori *12*: 387 (1927).

[71] H. Binhold: Ztschr. f. Krebsforsch. *38*: 587 (1933).

[72] Y. Nishiyama: Gann *31*: 223 (1937); *32*: 85 (1938).

[73] T. Nonaka: Gann *32*: 234 (1938).

[74] N. Takizawa: Gann *32*: 236 (1938); *33*: 193 (1939); *34*: 1 (1940).

[75] A. Zarattini: Tumori n. S. *14*: 77 (1940).

[76] E. G. Mayer: Klin. Wchnschr. *5*: 733; 1519 (1926); Fortschr. a. d. Geb. d. Roentgenol. *34*: 546 (1926).

[77] W. Raaflaub: Klin. Wchnschr. *6*: 89 (1927).

[78] K. Frik & K. Posener: Roentgenol. Kongr. Berlin 1926; Fortschr. Röntgenstr. *34*: 46 (1926).

[79] K. Inouye: Strahlentherapie *58*: 125 (1937).

[80] V. Czunft: Klin. Wchnschr. *5*: 1249 (1926).

[81] G. Holzknecht: Strahlentherapie *24*: 722 (1927).

[82] H. Gurniak: Strahlentherapie *24*: 750 (1927).

[82a] K. Naito, M. Segi, M. Ito & M. Miyabe: Monatschr. f. Krebsbekämpf. *9*: 32 (1941).

findings were also recorded.[83, 84] In connection with carbohydrate metabolism, the rationality of this treatment will be discussed, i.e., how an excess supply of glucose may influence the vitality of cancer cells (cf.[85]).

Pentoses. The presence of free pentoses has been detected in autolysates of liver metastases,[86] whereas free pentoses were lacking in normal organs as well as in the primary gastric cancer from which the said metastases originated.[87] The total pentose content of the tumors was determined as being approximately 1.7% of the dried tissue, whereas the values for liver and other normal organs, excepting pancreas, did not exceed one per cent.[87] Further studies showed an increased pentose content in breast cancers also, but in some other tumors only concentrations corresponding to that of the matrix were revealed.[88] It may be assumed that these findings depend on the increased nucleic acid content of some tumors, since in the animal body pentoses occur mainly in these compounds. The presence of free pentoses, mentioned above, points to autolytic decomposition of nucleic acids with consecutive liberation of their carbohydrate constituents. Intraperitoneal injections of gum arabic, a natural substrate rich in pentoses, slightly inhibited the takes of inoculated Flexner-Jobling carcinoma. Developed tumors also responded to such treatment by growth retardation. This action was explained by the swelling effect of gum arabic creating unfavorable conditions for tumor growth.[89]

Pyruvic acid. This compound is a product of intermediate carbohydrate metabolism. Earlier assumptions, identifying

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83. H. Füllsack: Strahlentherapie *28*: 795 (1928).
84. H. Schumacher: Klin. Wchnschr. *7*: 1773 (1928); *8*: 585 (1929).
85. M. Reiss: 1st Internat. Cancer Congress Madrid, Vol. II, p. 410 (1933).
86. C. Neuberg & R. Milchner: Berlin. klin. Wchnschr. *41*: 1081 (1904).
87. C. Neuberg: Berlin. klin. Wchnschr. *42*: 118 (1905).
88. S. P. Beebe & P. Shaffer: Am. J. Physiol. *14*: 231 (1905).
89. W. G. Hasselgreen: Ztschr. f. Krebsforsch. *45*: 495 (1937).

coenzyme T, a thermostable activator of glycolysis, obtained from tumors, with pyruvic acid,[90, 91] were later disproved.[92] An elevated concentration of pyruvic acid has been found in the blood of rats inoculated with Jensen sarcoma.[93] Although no pertinent explanation of this finding could be offered, its correlation with some oxidation disturbances was made probable by the observation that the high values were reduced almost to the norm by injections of nicotinic acid, cozymase and adenosinetriphosphate,[94] all of them intimately connected with the oxidation of sugar.

Lactic acid. A great deal of material has been contributed to the knowledge of the relationship between this important product of carbohydrate metabolism and tumor pathology; but most of these studies are closely connected with the metabolic studies of Warburg, which are discussed later. Here, only brief mention of one result of this work will be made, that is, the high capacity for lactic acid formation, glycolysis, inherent in malignant neoplasms. Even prior to the publications of Warburg, an abnormally high lactic acid concentration was found in malignant tumors, reaching approximately twice the amount of the lactic acid content of the healthy parental tissue.[95] A lactic acid accumulation was also noted in uterine cancer; 0.4 per cent as compared with 0.17 per cent in the normal organ.[25] Similarly, comparative analyses of benign and malignant tumors showed a definite prevalence of lactic acid in the latter.[9] As to the significance of these findings, a far reaching claim has been raised by a theory

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[90] B. Mendel: Klin. Wchnschr. *10*: 118 (1931).

[91] E. Bumm, H. Appel & P. Couceiro: Ztschr. f. physiol. Chem. *220*: 186 (1933).

[92] H. Kraut & R. W. Nefflen: Ztschr. f. physiol. Chem. *232*: 276 (1935).

[93] H. v. Euler, B. Skarżyński & B. Hoegberg: Ztschr. f. Krebsforsch. *50*: 230 (1940).

[94] H. v. Euler & I. Petterson: Ztschr. f. Krebsforsch. *51*: 193 (1941).

[95] R. Bierich: München. med. Wchnschr. *70*: 1145 (1923); Ztschr. f. physiol. Chem. *155*: 245 (1926).

which assumed that the augmented lactic acid was directly responsible for the invasive and destructive growth of malignant neoplasms. This, it was alleged, was brought about by a dehydrating action of the lactic acid on the surrounding connective tissue thus preparing it for the tumer cells, facilitating their penetrating growth.[96] The validity of this conception has been discredited by later work which definitely proved that the lactic acid formed in the tumor did not permeate into the surrounding tissue, and therefore it could not be connected with the penetrating tumor growth.[97] However, it has been ascertained that lactates do activate proteolytic enzymes,[98, 99] and thus may partly be responsible for the energetic protein metabolism of tumors. In view of the fact that the enzymatic processes of protein decomposition and of protein formation have been proven reversible, it is obvious that lactates may also be an important factor in the facilitating of protein formation by creating a favorable medium.[100]

Taking into consideration the increased lactic acid production by tumors, it was suggestive to investigate the lactic acid level of the blood of tumor patients. In fact, a rising of the lactic acid values to double the normal was reported in advanced cancer cases,[101] and similar hyperlacticemias of cancer patients were observed by other authors.[102-107] How-

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96. R. Bierich & A. Rosenbohm: Biochem. Ztschr. *152*: 193 (1924).

97. C. Voegtlin, R. H. Fitch, H. Kahler, J. M. Thompson & J. W. Thompson: Nat. Inst. Health Bull. No. 164, 1 (1935).

98. W. M. Rubel: Biochem. Ztschr. *283*: 180 (1936).

99. A. Rosenbohm: Biochem. Ztschr. *289*: 279 (1937).

100. M. E. Maver, J. M. Johnson & C. Voegtlin: Nat. Inst. Health Bull. No. 164, 29 (1935).

101. A. H. Roffo & C. Griot: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *3*: 373 (1927).

102. E. Liverani: Arch. di farmacol. sper. *51*: 177 (1931); abstr. Ztschr. f. Krebsforsch. *38*: Ref. 31 (1932).

103. J. A. Collazo, J. Puyal & I. Torres: Arch. españ. oncol. *3*: 21 (1932); abstr. Ztschr. f. Krebsforsch. *38*: Ref. 53 (1932).

104. F. Valentin: München. med. Wchnschr. *72*: 86 (1925).

ever, closer analysis showed that high lactic acid values generally accompanied liver metastases.[108–110] Hence the hyperlacticemia of cancer patients must rather be connected to a liver damage, caused by metastases or cachexia, than be considered a primary result of the cancerous process. In agreement with such a view, several authors obtained normal lactic acid values for the blood of non-cachectic tumor patients.[111–113] The ability of the normal liver to resynthesize comparatively large amounts of lactic acid has been demonstrated by the experiment of injecting 0.2 gm. of lactic acid intravenously into a dog, without causing a rise of the blood lactic acid level.[111] It must be borne in mind that such an amount of lactic acid would be produced, according to Warburg's results, by a tumor weighing approximately 600 gm.

Conflicting claims have been made regarding the action of roentgen and radium radiation on the lactic acid in the blood. One author observed a reduction of the lacticemia, ascribing this fact to an impairment of the tumor glycolysis caused by the radiation.[102] But another worker [114] reported that irradiation of malignant tumors resulted in a rise of the lactatemia, and he recommended such a procedure for diagnostic purposes. In view of the unspecific nature of a hyper-

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105. E. M. Gelstein & M. I. Frankstein: Ztschr. f. klin. Med. *111*: 563 (1929).

106. U. Fogliani: Riv. di pat. sper. *8*: 33 (1932); abstr. Am. J. Cancer *18*: 416 (1933).

107. F. Danmeyer, O. Hartleb & J. Schubert: Strahlentherapie *42*: 609 (1931).

108. O. Jervell: Norsk. Mag. Laegevidensk *91*: 528 (1930); abstr. Ztschr. f. Krebsforsch. *33*: Ref. 186 (1931).

109. H. Schumacher: Klin. Wchnschr. *5*: 497 (1926).

110. T. Kuzuhara: abstr. Am. J. Cancer *24*: 900 (1935).

111. B. Mendel & M. Bauch: Klin. Wchnschr. *5*: 1272 (1926).

112. H. E. Büttner: Klin. Wchnschr. *5*: 1507 (1926).

113. L. Bugnard, H. Colombies & J. Rogeon: Bull. Assoc. franç. p. l'étude du cancer *27*: 342 (1938).

114. J. Heeren & R. Hummel: Strahlentherapie *42*: 784 (1931).

lacticemia in cancer patients, as discussed above, no value can be attributed to these studies.

Lactic acid determinations in the spinal fluid of sixteen women suffering from genital cancer yielded elevated values.[64] Attempts to find lactic acid in the urine of tumor patients were made at an early date. Whereas at first negative results were obtained,[115] later on a few cases were reported to have shown the presence of lactic acid in the urine.[116] A considerable lactaturia was claimed as occurring in cancer patients following intravenous glucose injections,[117] but this finding has not been confirmed.[118] However, it was established that parenteral sugar administration in animal experiments caused lactic acid accumulation in the tumors.[119, 97] Provided that this excess of lactic acid is thrown into circulation (but in view of the relations discussed above, this is not at all certain), a lactate excretion in the urine would be facilitated, in agreement with the finding that tumor-bearing animals showed a decreased lactate tolerance; i.e., administration of small lactate amounts caused lactaturia, which had no such effect in normal animals.[120] Further studies of this problem would be desirable not only to substantiate these results but, also, to indicate whether they can be utilized for diagnostic purposes.

As a carcinogen, lactic acid was successfully employed only for producing plant tumors in carrots,[121] cabbage, and parsley,[122] respectively. The resemblance of the tumors thus obtained to those produced by Bacterium tumefaciens suggested that the latter were caused by similar products of bacterial metabolism.[123] It is, however, extremely doubtful whether any application of these results to animal and human tumors is justified, because of the tremendous biological differences between these categories of tumors.

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[115] K. Schütz: Ztschr. f. physiol. Chem. *19*: 482 (1894).

[116] K. v. Noorden: Lehrbuch d. Pathologie des Stoffwechsels, Berlin, 1903, p. 461.

[117] K. Glaessner: Wien. klin. Wchnschr. *37*: 358 (1924).

[118] R. Bauer & W. Nyiri: Wien. klin. Wchnschr. *38*: 853; 881 (1925).

[119] C. F. Cori: J. Biol. Chem. *65*: 11 (1925).

[120] C. F. Cori & G. T. Cori: J. Cancer Res. *11*: 190 (1927).

[121] F. Blumenthal: Med. Klin. *20*: 594 (1924); Ztschr. f. Krebsforsch. *21*: 250 (1924).

[122] O. Bittmann: Ztschr. f. Krebsforsch. *22*: 291 (1925).

A growth inhibition, and sometimes regression, of rat and fowl sarcoma was reported to have been achieved by means of prolonged injections of sodium lactate.[124, 125] However, analogous experiments performed with mouse tumors showed only a very slight growth retardation, and this effect was not correlated with a specific action of the lactic acid but, instead, was explained by the impaired general condition of the treated animals.[126, 127] Other authors even reported growth stimulation of transplanted mouse tumors following lactate injections,[128] and an increased virulence of rat tumors, when lactic acid had been added to the inoculated material.[129]

Other possible products of carbohydrate metabolism, including *citric acid,* were investigated and high values for this substance were found in various transplanted tumors, as well as in embryonic tissue and in newborn mice.[130]

Determinations of *oxalic acid* in the blood of cancer patients yielded mostly normal values although, sometimes, elevated values were found, but even in these cases no relation to the tumor process could be established.[46]

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[123] A. Kotzareff & R. Fischer: Les Cancers et la Physico-chimie, Paris, Vigot (1927).

[124] I. A. Parfentjev, W. Devrient & B. Sokoloff: Proc. Soc. Exper. Biol. & Med. *28*: 538 (1931).

[125] I. A. Parfentjev, W. Devrient, V. D. Suntzeff & B. Sokoloff: Am. J. Cancer *16*: 366 (1932).

[126] I. A. Parfentjev, V. D. Suntzeff & W. Devrient: Am. J. Cancer *20*: 117 (1934).

[127] W. R. Franks, M. M. Shaw & W. H. Dickson: Am. J. Cancer *22*: 601 (1934).

[128] B. Fischer-Wasels: Gastherapie bösartiger Geschwülste, Frankfurt a. M., 1929, p. 71.

[129] P. Rostock: Deutsche med. Wchnschr. *47*: 1323 (1929).

[130] F. Dickens: Chem. & Indust. *59*: 135 (1940); Biochem. J. *35*: 1011 (1941).

2. *Proteins.*

Chemical cancer research has been confronted with the protein problem from its very start, necessarily so, because this mysterious substance occupies the central position in every living cell. Therefore, consistent efforts have been made to reveal significant quantitative or qualitative changes of the tumor proteins, which could in turn offer a stepping stone to the solution of cancer etiology. True, the obstacles encountered on this path are partly inherent in the protein problem itself. The chemistry of proteins has progressed insufficiently to warrant an insight into this most important and most complicated phenomenon of biology. It must be admitted that almost all progress which had been made in protein biology, and every new chemical or physico-chemical method devised in this field, has been shortly afterward employed for the investigation of tumor proteins. If all this has been of no avail up to now, two factors may be held responsible; one, that in spite of the impressive advancement of protein chemistry and the increased reliability and efficiency of the methods developed, a true insight into the complicated protein structure has not yet been gained. Secondly, the failure of tumor protein chemistry to detect a basic difference from normal protein may give rise to the doubt as to whether this cell constituent is at all essentially involved in carcinogenesis and tumor development or, instead, whether other chemical constituents, perhaps lipids, play a more important part in this respect. In this case, structural changes of tumor proteins, as far as they might be established, would have to be considered as a secondary phenomenon.

Quantitative protein relations in tumors. Measurements of the total protein content of malignant tumors showed a considerable diminution of heat-coagulable substance, contrasted to an increased incoagulable nitrogen, and an augmentation of the nucleoprotein fraction.[131] The latter finding may be attributed to the increased nuclear content of tumors, whereas the raised incoagulable nitrogen content, obviously caused at the expense of the coagulable protein, was considered as an expression of energetic enzymatic, protein-splitting processes. However the question was also raised as to whether the diminished protein content of tumors could not be explained by their higher water content.[132] But, at least for some animal tumors, such an explanation does not appear to be valid. The protein-nitrogen of mouse carcinoma was determined as 40–50 mg. per cent, as compared with values of 80–85 mg. per cent for the healthy leg muscle, and this difference of almost 100 per cent cannot be accounted for by the variation in the water content which was found to be twenty-five per cent in favor of the tumor. In mouse sarcoma, however, the total protein nitrogen was determined as 90–100 mg. per cent, which is in excess of the values of normal muscle.[133]

In extracts of both normal organs and malignant tumors, quantitative analyses of the protein fractions, characterized as to their molecular size, have been performed.[134] Whereas in the normal organs the globulin and the albumin fractions yielded approximately equal amounts, in the tumor extracts a prevalence of the albumin fraction was found, and within the globulin fraction the pseudoglobulin was increased at the expense of the euglobulin. Thus one would be justified in speaking of a shift of the tumor proteins to the side of the

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131. E. Petry: Ztschr. f. physiol. Chem. *27*: 398 (1899); Hofmeister's Beitr. *2*: 94 (1902).
132. C. Neuberg: Oppenheimer's Handbuch d. Biochemie, *4*: 464.
133. A. Goldfeder: Acta Cancrol. *2*: 333 (1936).
134. H. Wolff: Ztschr. f. Krebsforsch. *3*: 95 (1905).

fractions with lower molecular weight. Deviations of the protein factions, as compared with normal organs, have also been observed in the rat Jensen sarcoma.[135] In this tumor the proteins which can be extracted by means of a 1% saline solution were diminished to 4.6 per cent of the fresh weight while, for instance in the liver, they amounted to fourteen per cent. Furthermore, a decrease of the high-molecular protein fractions, which had been observed after roentgen radiation of normal tissues, failed to occur in the tumors. Quantitative differences distinguishing protein fractions of Jensen rat sarcoma from normal organs were also revealed in a study comparing saline and alkaline extracts of these tissues.[136] The tumor proved to be richer in the latter protein fractions and they exhibited a higher phosphorus content than those obtained from normal tissue.

Chemical structure of tumor proteins. The work done on this subject was originally based on the fundamental achievements of E. Fischer, who devised methods for the chemical analysis and quantitative measurement of the amino acids composing the protein molecule. By means of his esterification method, a vast number of plant and animal proteins have been successfully analyzed and, consequently, this method was employed in tumor chemistry. At first negative results were obtained, inasmuch as the analysis of five animal tumors demonstrated normal values of glycine, glutamic acid and of tyrosine.[137] Investigations, however, dealing with the diamino acids and the hexone bases, respectively, led to more revealing results. An investigation of mouse tumors indicated a very high content of diamino acids, both when com-

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135. E. G. Schenck: Arch. f. exper. Path. u. Pharmakol. *175*: 401 (1934).

136. P. Rondoni: Ztschr. f. physiol. Chem. *265*: 102 (1940).

137. E. Abderhalden & F. Medigreceanu: Ztschr. f. physiol. Chem. *69*: 66 (1910).

pared with organs of normal animals, or with the organs of the tumor hosts.[138] The analysis of hydrolysates of five malignant tumors showed their concentration of hexone bases (lysine, histidine, arginine) to attain more than twice the values of normal tissues.[139] A similar increase of diamino acids was observed in carcinoma autolysates.[140] The accumulation of these substances in tumors has been connected with their great nuclear content,[141] i.e., with their mitotic activity. Actually, an increased content of diamino acids was also found in other rapidly growing tissues.[142] This is in satisfactory agreement with the work of Osborne and Mendel[143] who proved some of these substances were important growth factors. Hence the established richness of tumors in hexone bases and diamino acids cannot be considered as characteristic of malignancy, but must be viewed as accompanying rapid growth processes. Divergent results have been reported regarding the Jensen rat sarcoma. In this tumor, and to a slighter degree also in the liver, kidney and muscle of tumor-bearing animals, an increase of arginine, and a decrease of the lysine content were observed, leaving the total amount of hexone bases unchanged, as compared with normal organs.[135] Other authors[144] found the total arginine content of mouse carcinoma, rat sarcoma and Rous sarcoma unchanged, but a deviation in the distribution between protein-bound and free arginine was detected; the latter having been increased at the expense of the former.[144] In another investigation of the Jensen sarcoma, however, both an increase of the free arginine (38.5 mg./kg. as compared with 14.7 mg./kg. in fresh liver), as well as an increase of the total arginine (4.46 per cent of dried tumor, 3.16 per cent of dried liver) have been reported.[145] Comparison of the hexone bases content in the necrotic and in the non-necrotic parts of Jensen sarcoma did not reveal any difference.[146]

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[138] P. Bergell: Ztschr. f. Krebsforsch. *5*: 204 (1907).
[139] B. A. Kocher: J. Biol. Chem. *22*: 285 (1915).
[140] S. Yoshimoto: Biochem. Ztschr. *22*: 289 (1909).
[141] J. C. Drummond: Biochem. J. *10*: 473 (1916).
[142] Y. Washizu: Mitt. a. d. med. Akad. zu Kioto *20*: 1591 (1937).
[143] T. B. Osborne & L. B. Mendel: J. Biol. Chem. *12*: 472 (1912); *13*: 233 (1913); *17*: 325 (1913).
[144] G. Klein & W. Ziese: Ztschr. f. Krebsforsch. *37*: 323 (1932).

Tryptophane. Different tumors apparently do not exhibit a uniform behavior as to their content of this protein component. Whereas skin cancers, and also liver metastases, were reported to show a high tryptophane content, it was found completely lacking in breast cancer.[147] The validity of an earlier statement, describing a tryptophane diminution in skin cancer also,[148] has been rejected because of the use of allegedly unsuitable tumor material.[147] Later studies indicated a varying, but generally low tryptophane concentration in rat tumors. No difference of the tryptophane content was found to exist between organs of tumor-free and tumor-bearing rats.[149] The low tryptophane content of Jensen rat sarcoma, corresponding to that of normal organs, was confirmed,[150, 151] but divergent opinions have been expressed regarding the comparative analysis of necrotic and non-necrotic tumor parts. Technical differences may account for the fact that one study did not reveal any difference in this respect,[150] whereas another claimed a relative tryptophane increase for the necrotic tumor areas.[151]

Investigations of the tryptophane and tyrosine content of carcinoma of the biliary duct and of liver metastases did not indicate any deviation from the quantitative relations in the

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145. E. Annau & B. Gözsy: Ztschr. f. Krebsforsch. *40*: 572 (1934).

146. S. Edlbacher & W. Baúmann: Ztschr. f. Krebsforsch. *44*: 441 (1936).

147. H. Fasal: Biochem. Ztschr. *55*: 88 (1913).

148. S. Fränkel: Wien. klin. Wchnschr. *25*: 1041 (1912).

149. A. Rosenbohm: Ztschr. f. Krebsforsch. *48*: 117 (1938).

150. S. Edlbacher & W. Baumann: Ztschr. f. Krebsforsch. *47*: 198 (1938).

151. A. Lang: Ztschr. f. Krebsforsch. *48*: 29 (1938).

surrounding healthy tissue. Melanosarcoma metastases of the liver, however, showed almost normal values of tyrosine, but a considerable reduction of the tryptophane content (0.6 per cent–1.5 per cent, as compared with 2.2 per cent–3.3 per cent in normal liver).[152] This finding was viewed as corroborating the conception that tryptophane, and not tyrosine, should be considered the parent substance of the melanotic tumor pigments.[153]

While it is obvious that the studies enumerated did not succeed in establishing a characteristic chemical deviation of the tumor proteins, a new approach to this problem has been attempted in the work of Kögl and his collaborators. These authors [154–157] announced that polarimetric analysis of protein hydrolysates, and of isolated amino acids obtained from malignant tumors, revealed a striking divergence from the normal, insofar as the presence of a considerable amount of racemic amino acids was observed. Analyzing a variety of human and animal neoplasms, the authors found particularly large quantities of d(—)-glutamic acid. Isolation of pure crystals of this substance appeared to substantiate these results. No corresponding findings were obtained by investigation of normal or embryonic tissue, of benign tumors or otherwise diseased organs, such as necrotic kidney, liver cirrhosis and pulmonary tuberculosis.[158] These reports naturally evoked the greatest interest and extensive re-examination. However, of these subsequent studies, only compara-

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152. O. v. Fürth, H. Kaunitz & F. Scherf: Biochem. Ztschr. *272*: 88 (1934).

153. H. Eppinger: Biochem. Ztschr. *28*: 181 (1910).

154. F. Kögl & H. Erxleben: Ztschr. f. physiol. Chem. *258*: 57 (1939).

155. F. Kögl, H. Erxleben & A. M. Akkerman: Ztschr. f. physiol. Chem. *261*: 141 (1939).

156. F. Kögl & H. Erxleben: Ztschr. f. physiol. Chem. *261*: 154 (1939).

157. F. Kögl: Ztschr. f. Krebsforsch. *49*: 291 (1939).

158. H. Erxleben & H. Herken: Ztschr. f. physiol. Chem. *264*: 240 (1940).

tively few were able to arrive at confirmatory results, i.e., to prove the presence of considerable amounts of d-glutamic acid, or other d-amino acids, in malignant tumors of human or animal origin.[159–162] In contrast to these reports, the majority of workers [163–171] found themselves obliged, on the basis of their analyses, to discount the validity of Kögl's statements regarding the configuration of amino acids in malignant tumors. Either no, or only insignificant amounts of racemic amino acids, values not differing from those in normal tissues, were detected in malignant tumors by these authors. Kögl [172] attempted to discredit some of these re-examinations by claiming that deviations in technique from his original method of the technique employed were responsible for these conflicting results, a conception which seemed to be supported by the work of other authors; [159] but exact analyses using the same method as Kögl sustained the earlier negative findings.[166] In particular, Kögl [173] took exception

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159. J. White & F. R. White: J. Biol. Chem. *130*: 435 (1939).
160. L. E. Arnow & J. C. Opsahl · Science *90*: 257 (1939).
161. L. E. Arnow, J. C. Opsahl & C. J. Watson: Proc. Soc. Exper. Biol. & Med. *43*: 766 (1940).
162. H. Ottawa: Ztschr. f. Krebsforsch. *49*: 677 (1940).
163. S. Graff: J. Biol. Chem. *130*: 13 (1939).
164. A. C. Chibnall, M. W. Rees, G. R. Tristram, E. F. Williams & E. Boyland: Nature, London *144*: 71 (1939).
165. J. M. Johnson: J. Biol. Chem. *132*: 78 (1940); J. Nat. Cancer Inst. *1*: 113 (1940).
166. S. Graff, D. Rittenberg & G. L. Foster: J. Biol. Chem. *133*: 745 (1940).
167. A. Konikova: Nature, London *145*: 312 (1940).
168. O. K. Behrens, F. Lipmann, M. Cohn & D. Burk: Science *92*: 32 (1940).
169. A. C. Chibnall, M. W. Rees, E. F. Williams & E. Boyland: Biochem. J. *34*: 285 (1940).
170. R. G. Mathers: Cancer Res. *1*: 146 (1941).
171. C. Dittmar: Ztschr. f. Krebsforsch. *49*: 397 (1939).
171a. G. E. Woodward, F. E. Reinhart & J. S. Dohan: J. Biol. Chem. *138*: 677 (1941).
172. F. Kögl & H. Erxleben: Ztschr. f. physiol. Chem. *264*: 198 (1940).
173. F. Kögl, H. Herken & H. Erxleben: Ztschr. f. physiol. Chem. *264*: 220 (1940).

to a study which used an indirect method of determining d-amino acids by means of the specific enzyme d-amino oxidase.[168] But even if the validity of this result is considered doubtful, the assumption of a specific occurrence of d-amino acids in malignant neoplasms appears unwarranted. Some observations were made indicating the possibility of a formation of d-glutamic acid from the l-compound during hydrolysis, and hence the presence of the d-amino acids would be an artifact.[174] An attempt has been made to explain the divergent results by assuming that d-amino acids occurred only in necrotic tumor parts, and were formed there by secondary (perhaps bacterial) changes,[175] but the same author [176] abandoned this opinion on the basis of further studies in which he also found necrotic human and animal tumors devoid of d-amino acids. In view of the definitely unclear state of this problem, reference to the theoretical conceptions of Kögl[153-157] which envisaged the presence of d-amino acids as a factor of utmost importance for tumor pathology involving enzymatic and metabolic anomalies may be omitted. Enzymatic studies which bear on this question will be discussed in their proper place.

Supplementing this discussion of the tumor protein structure, it must be mentioned that in addition to chemical and physico-chemical methods, immunologic methods have also been employed for the elucidation of this problem. It is true, the latter approach had been proved the most useful in biology, in instances where the purely chemical methods had been found insufficient. But as far as tumor chemistry is concerned, no convincing success has been achieved thus far. An older study, employing precipitin tests, led to the rather quaint theory that tumor proteins corresponded to proteins

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174. J. M. Johnson: J. Biol. Chem. *134*: 459 (1940).
175. C. Dittmar: Ztschr. f. Krebsforsch. *49*: 441 (1939).
176. C. Dittmar: Ztschr. f. Krebsforsch. *50*: 472 (1940).

foreign to the species, i.e., to embryonic proteins of various animals,[177] but this claim was soon rejected as lacking an adequate theoretical and experimental foundation.[178] On the basis of anaphylactic reactions, it has been assumed that an identity of the tumor proteins with the serum albumin of the tumor host could be proved. Hence, it was claimed that the serum albumin represented the main source of the tumor proteins; [179] confirmatory studies on this question are not available. Additional work dealing with immunologic properties of tumor proteins will be discussed in Chapter VIII.

Protein relations in the blood serum of the tumor host. According to a great number of investigations dealing with the quantitative analysis of the blood serum proteins in tumor hosts, an absolute diminution of these substances is found, in analogy with the relations observed in the neoplasms themselves, but regarding the molecular size, the opposite behavior was noticeable, i.e., an increase of the globulins of higher molecular weight and a decrease of the albumin fraction of lower molecular weight (diminution of the albumin/globulin-ratio). These results were obtained in clinical material [180–195] and in animal experiments.[194, 196, 197] However, ad-

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177. G. Kelling: Berlin. klin. Wchnschr. *42*: 950 (1905).
178. E. v. Dungern: Ztschr. f. Krebsforsch. *5*: 48 (1907).
179. M. Loeper & J. Tonnet: Compt. rend. Soc. de biol. *83*: 1032 (1920).
180. M. Loeper & J. Tonnet: Compt. rend. Soc. de biol. *83*: 1086 (1920).
181. N. Louros & Gaessler: Ztschr. f. Krebsforsch. *28*: 191 (1927).
182. O. Galehr: Wien. Arch. f. inn. Med. *9*: 379 (1924).
183. H. Kahn: Ergebn. d. inn. Med. u. Kinderh. *27*: 186 (1925).
184. S. Rusznyak, I. Barát & L. Kürthy: Ztschr. f. klin. Med. *98*: 337 (1924).
185. W. Starlinger & E. Winands: Ztschr. f. d. ges. exper. Med. *60*: 185 (1928).
186. W. Baer: Klin. Wchnschr. *6*: 2380 (1927).
187. F. Rohrer: Deutsches Arch. f. klin. Med. *121*: 221 (1916).
188. S. Gussio. Tumori *10*: 1 (1923).
189. H. Guthmann & H. G. Plotz: Arch. f. Gynäk. *141*: 557 (1930).
190. C. Loebner: Deutsches Arch. f. klin. Med. *127*: 397 (1918).
191. G. Lewin: Med. Klin. *23*: 643 (1927).
192. G. Ferdinandy: Deutsche med. Wchnschr. *59*: 472 (1933).

ditional studies clearly indicated that these changes of the serum proteins in neoplasia are not at all constant. In a great percentage of sera tested, the rise of the globulin fraction was not detected and, moreover, uncharacteristic oscillations of the protein concentrations were frequently observed.[198, 199] Other workers showed that the diminished albumin and the increased globulin concentrations, observed in the majority of the cancer sera, lie just on the lower, or the upper level, respectively, of the norm.[200] Doubtless, these divergencies must be considered as being caused by differences of the material investigated, as to localization and advancement of the neoplasms, and as to secondary complications. On the basis of a review of the literature, it was concluded that the shift observed of the albumin globulin ratio in sera of tumor-bearers did not by any means represent a phenomenon specific for neoplastic disease, but may be also noted, in a similar manner, in various inflammatory or degenerative conditions;[201] in particular it was connected with processes involving tissue destruction and cachexia.[191]

As far as other protein fractions are concerned, numerous investigations furnished proof of a marked increase of fibrinogen in the blood of tumor hosts,[202-206, 181, 185, 193] thus con-

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193. A. Goldfeder: Ztschr. f. Krebsforsch. *40*:399 (1934).
194. B. Purjesz: Ztschr. f. Krebsforsch. *44*: 1 (1936).
195. S. Marino & D. Trapani: Riforma med. *52*: 423 (1936).
196. S. L. Malowan: Biochem. Ztschr. *264*: 224 (1933).
197. W. Raab: Klin. Wchnschr. *14*: 1633 (1935).
198. C. Dalla Rosa: Arch. di pat. e clin. med. *2*: 614 (1923).
199. E. L. Kennaway: Quarterly J. Med. *17*: 302 (1924).
200. J. Ducuing & Miletzky: 1st Internat. Cancer Congress, Madrid, : 494 (1933).
201. E. Boyland: Cancer Review *7*: 433 (1932).
202. O. Weltmann & K. Neumayer: Med. Klin. *21*: 629 (1925).
203. P. Benedetti: Ronas Ber. *32*: 876 (1925).
204. G. Lewin: Med. Klin. *23*: 623 (1927).
205. H. C. Gram: Acta med. Scandinav. *56*: 107 (1922).
206. R. Loicq: Compt. rend. Soc. de biol. *120*: 253 (1935).

firming the shift of the blood proteins to the higher-molecular phase. In addition, this was corroborated by studies dealing with the colloidal qualities of the albumin fractions. By means of various methods, it has been demonstrated that the so-called albumin A-fraction, which is not precipitated by a 37.15 per cent ammonium sulfate solution is either missing or markedly diminished in sera of cancer patients, and in tumor-bearing animals.[188] In spite of the fact that the author proposed and employed a method based on these findings for diagnostic purposes, he did not assume that these or other deviations of the serum protein distribution were specific for malignant tumors, but they may likewise be seen in other pathologies, such as liver damage, kidney affections, and some infectious diseases. This tallies with the results of other work which led to the conclusion that the decreased albumin, the increased globulin and fibrinogen contents observed in the blood of cancer patients are due to the increased protein resorption caused by tissue breakdown.[207] Hence the described anomalies of the blood protein distribution cannot be considered as primarily depending on the neoplastic process.

It must, however, be kept in mind that a shift of the albumin/globulin ratio is not merely a phenomenon of colloid-chemical changes, but it also entails chemical differences, since these two protein fractions have been found to be distinguished from each other by their glycine content,[208] by the number of end amino groups,[209] and by their content of histidine and tryptophane.[210] As to the latter amino acid, it has been reported that the serum albumin of cancer patients

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207. R. Reding: Compt. rend. Soc. de biol. *123*: 1238 (1936).

208. E. Abderhalden: Ztschr. f. physiol. Chem. *37*: 499; *39*: 143 (1903).

209. F. Obermayer & R. Willheim: Biochem. Ztschr. *38*: 331 (1912); *50*: 369 (1913).

210. S. Goldschmidt & H. Kahn: Ztschr. f. physiol. Chem. *183*: 19 (1929).

showed a decreased tryptophane content,[211] and this was confirmed particularly for the albumin II fraction in the blood of cancer patients.[212] In rats inoculated with various tumors (Jensen sarcoma, Flexner-Jobling carcinoma, Walker sarcoma), however, the tryptophane content of serum albumin II was found somewhat increased, and this finding was correlated with decomposition and diffusion of tumor proteins.[213] An attempt to characterize the serum protein of cancer patients by means of measuring the amide nitrogen (nitrogen which is split off as ammonia after twenty hours of hydrolysis with 20 per cent hydrochloric acid) [214] failed, in continued studies, to reveal any significant difference from the normal.[215, 216] A deviation of the cystine distribution was observed in carcinomatous and sarcomatous exudates where the euglobulin fraction was found to be richer by ten to twelve per cent in cystine, the albumin fifteen to twenty-five per cent poorer in cystine than the proteins concerned in exudates of non-malignant origin.[217] The protein composition of the stomach contents of patients suffering from gastric cancer exhibited a difference from gastric juices of normal or otherwise diseased persons, by the presence of protein which can be precipitated by phosphotungstic acid. It was assumed that this protein was derived by autolysis from the gastric cancer.[218, 219]

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[211] A. Fischer & H. Weiss: Ztschr. f. d. ges. exper. Med. *48*: 111 (1926).

[212] R. Bierich & A. Lang: Biochem. Ztschr. *284*: 443 (1936).

[213] A. Rosenbohm: Ztschr. f. Krebsforsch. *49*: 665 (1940).

[214] P. F. Merzbach: Klin. Wchnschr. *11*: 1984 (1932).

[215] P. F. Merzbach: Frankfurt. Ztschr. f. Path. *47*: 116 (1934).

[216] W. Schlegel: Erlangen, Diss. 1935; abstr. Ztschr. f. Krebsforsch. *44*: Ref. 201 (1936).

[217] B. Lustig & E. Mandler: Biochem. Ztschr. *278*: 312 (1935).

[218] W. Wolff & P. Junghans: Berlin. klin. Wchnschr. *48*: 978 (1911).

[219] C. S. Hicks: Lancet *2*: 546 (1924).

Colloid chemical studies of the serum proteins in malignancy were performed by nephelometric measurements of precipitates produced by ammonium sulfate solutions of varying concentrations and by charting adequate graphs from the readings obtained. These curves showed a form, differing from the normal, when the sera were derived from cancer patients (changes of the "albumin-peak"), but as similar deviations were noted with sera of tuberculous patients, this phenomenon could not be considered as specific for malignancy.[220] The spectrographic analysis of the protein fractions of the blood plasma was reported to reveal differences of the absorption spectra between tumor-free and tumor-affected humans or rats,[221] but plasma of normal and of tumor-immune rats could not be differentiated by this method.[222] This work, however, does not seem to have been continued to the extent which would make possible an exact evaluation.

Protein cleavage products: proteoses and peptones. Only few papers dealt with the presence of these substances in tumors. In cancer autolysates the proteoses-nitrogen was found to be diminished, whereas increased values were obtained for the nitrogen of a fraction comprising peptone, diamino acids and ammonia.[140] On the basis of previously discussed results, it appears likely that this increase was mainly due to a diamino acid augmentation. According to the theory of Freund [223] peptone plays an important part in the formation of sarcomas. He found a high capacity of sarcoma cells to adsorb peptone in vitro and observed an increased peptone content of the blood serum of sarcoma patients.[83] Pulmonary sarcoma was demonstrated to be especially rich in peptones and proteoses.[10]

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[220] A. Schmitz & F. Wulkow: Biochem. Ztschr. *245*: 408 (1932).

[221] A. J. Allen, R. G. Franklin & E. B. Sanigar: Am. J. Cancer *27*: 296; 301 (1936).

[222] R. G. Franklin, L. Smith & A. J. Allen: Am. J. Cancer *29*: 341 (1937).

[223] E. Freund & G. Kaminer: Biochemische Grundlagen der Karzinomdisposition, Wien, Springer, 1925.

The polypeptide-nitrogen in mouse carcinoma and sarcoma has been determined approximately equal to the values of normal muscle.[138] A hyperpolypeptidemia of cancer patients has been reported,[224, 225] but this finding was by no means regular.[226] It was observed by one author [227] in the early stage of a transplanted guinea pig liposarcoma, but was not found in cancer patients. In these persons it occurred to a marked degree only when liver damage was present [228] or after roentgen radiation.[229] Therefore, tissue destruction was generally believed to be responsible for its occurrence, as was convincingly shown in tumor-bearing dogs.[230]

A considerable amount of work has been devoted to one polypeptide, the tripeptide *glutathione.* Technical difficulties, and the variety of the methods employed, seem to be at least partly responsible for the conflicting results which have been reported regarding the glutathione concentration in malignant tumors. In rat carcinoma and sarcoma, values were found which correspond approximately to those in liver, which latter exhibits the highest glutathione content among normal organs; necrotic tumor portions were found to contain no glutathione.[231] Analysis of the Rous sarcoma showed that this tumor contains about the same glutathione amounts as the surrounding normal tissue.[232] Similarly, investigations of

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[224] A. Hahn: Berlin. klin. Wchnschr. *58*: 802 (1921).

[225] F. Ramond & P. Zizine: Compt. rend. Soc. de biol. *87*: 657 (1922).

[226] P. Duval, J. C. Roux & Goiffon: Presse méd. *44*: 41 (1936).

[227] R. Loicq: Compt. rend. Soc. de biol. *120*: 250 (1935).

[228] M. Tabanelli: Atti e Mem. soc. lomb. chir. *4*: 133 (1936) abstr. Ztschr. f. Krebsforsch. *44*: Ref. 134 (1936).

[229] M. Cellina: Giorn. clin. med. *17*: 909 (1936).

[230] F. Liégois & P. Térache: Compt. rend. Soc. de biol. *124*: (1937).

[231] C. Voegtlin & J. W. Thompson: J. Biol. Chem. *70*: 801 (1926).

[232] E. L. Kennaway & I. Hieger: Biochem. J. *21*: 751 (1927).

twenty-four gynecologic human neoplasms yielded high glutathione values which, however, did not differ significantly from the values of the matrix, and which were exceeded by those of adrenals, kidney and liver.[233] Some studies even claimed a lowered glutathione concentration for malignant tumors. Thus very low values of reduced glutathione were obtained for a rat carcinoma,[234] and analogous findings were reported for Rous sarcoma.[235] Histochemical estimations led to the conclusion that epitheliomas contained rather less glutathione than normal epithelium,[236] and a diminished glutathione content was observed in Ehrlich carcinoma of mice, Rous fowl sarcoma and in some human neoplasms.[237] The glutathione content of uterine cancer was determined with varying results, but generally the oxidized form was found to prevail.[238] Thus, it is generally implied that the total glutathione content of malignant tumors is certainly not significantly increased, and that the oxidized form (—S—S—) prevails over the reduced form (containing the sulfhydryl group (—SH)).[231] These findings therefore raise grave doubts as to the validity of an older statement according to which, in malignant tumors, the reduced form exceeds the oxidized one, a relation which may be expressed by the ratio

$$\frac{\text{Reduced glutathione}}{\text{Oxidized glutathione}}.$$

This ratio was claimed as being less than one in normal tissues, but greater than one in malignant tumors.[239]

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[233] P. Castagna: Arch. ostetr. *41*: 224 (1934); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 71 (1935).

[234] B. E. Holmes: Biochem. J. *20*: 812 (1926).

[235] H. Yaoi & W. Nakahara: Proc. Imp. Acad. Tokyo *2*: 449 (1926).

[236] V. Jura: Policlinico (sez. chir.) *37*: 372 (1930).

[237] H. Heinlein: Ztschr. f. Krebsforsch. *35*: 161 (1932).

[238] B. Maximowitsch: Akuš. i Ginek. *10*: 1229 (1936); abstr. Ztschr. f. Krebsforsch. *45*: Ref. 237, (1937).

[239] R. Bierich & K. Kalle: Ztschr. f. physiol. Chem. *158*: 1 (1926).

Since later work could not confirm such a relationship, the foundation has been withdrawn from a theory which ascribed great significance for tumor pathology to the reduced glutathione as an activator of proteolytic enzymes.[240, 241] In a similar manner, it was presumed that lactic acid, formed by the tumor, should increase the amount of reduced glutathione, and hence activate the cathepsin in tumor tissue.[99] The value of many of the investigations mentioned appears to be impaired by the fact that iodometric, or other nonspecific methods, were employed for the glutathione determination. Therefore, especial significance must be attributed to a study in which the glutathione content of rat tumors was measured by means of the specific method of glyoxalase activation, thus yielding values which were equal to thirty to fifty per cent of those in the liver.[242] Hence, these results, obtained with a reliable method, also indicate a somewhat decreased glutathione content of malignant tumors.

According to some publications, this glutathione deficiency seems to extend to the whole body of the tumor host, since a decrease, parallel to the tumor advancement was found in the other tissue.[231, 243] This phenomenon was most marked in thyroid and adrenals, and least marked in spleen.[244] A moderate glutathione decrease was noted in the liver of rabbits and rats, following the intraperitoneal injection of the carcinogen dibenzanthracene.[245] However, because of great variations in the glutathione values determined, the significance of these findings must not be overrated.

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240. E. Waldschmidt-Leitz & A. Schäffner: Naturwiss. 1930: 280.

241. E. Waldschmidt-Leitz, A. Purr & A. K. Balls: Naturwiss. 1930: 644.

242. G. E. Woodward: J. Biol. Chem. *109*: 1 (1935); Biochem. J. *29*: 2405 (1935).

243. L. Kyyanovskay: Bull. biol. et méd. expér. URSS *7*: 91 (1939).

244. W. Medvedeva: Z. med. Ciklu *2*: 93 (1932); abstr. Ztschr. f. Krebsforsch. *37*: Ref. 21 (1932).

245. A. Goerner & M. M. Goerner: Am. J. Cancer *37*: 518 (1939).

A diminished glutathione content was repeatedly reported for the blood of cancer patients [246–249] as well as of rabbits affected with tar carcinoma.[249] By correlating the glutathione values with the erythrocytes, an index was obtained which was significantly lower than that of cancer-free persons,[247] whereas cancer-free persons, suffering from anemia, showed an opposite behavior.[250] However, in disagreement with these findings, other authors observed normal glutathione values in the blood of cancer patients,[251] or found the glutathione decrease corresponding to the anemia.[252] No characteristic changes of the glutathione level were observed in the blood of Rous sarcoma fowl [44] and of rabbits inoculated with sarcoma [253] or carcinoma.[248]

Free amino acids. These elementary components of the protein molecule are generally not present in large amounts in normal tissues, nor in malignant tumors. The free amino-acid nitrogen has been determined in mouse carcinoma as being 30–82 mg. per cent, in mouse sarcoma as being 70–80 mg. per cent, as compared with values of 65–70 mg. per cent for normal muscle.[183] As mentioned above, a rise of the free arginine was reported as occuring in mouse carcinoma, rat sarcoma and fowl sarcoma.[144] Free cysteine could not be demonstrated in malignant tumors nor in normal tissues, excepting kidney, provided that the tissues were subjected rapidly to fixation after their removal from the body.[254]

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246. F. F. Ulibarri: Arch. españ. oncol. *1*: 349 (1930).
247. R. Willheim & K. Stern: Biochem. Ztschr. *260*: 180 (1933).
248. R. Truhaut & E. G. Minopoulos: Bull. Assoc. franç. p. l'étude du cancer *21*: 717 (1932).
249. S. F. Gomes da Costa: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *12*: 619 (1935).
250. I. Bach & E. Bach: Biochem. Ztschr. *236*: 174 (1931).
251. J. W. Schoonover: Am. J. Cancer *23*: 311; 315 (1935).
252. A. E. Osterberg, R. J. Coffey, J. A. Bargen & C. F. Dixon: Am. J. Cancer *32*: 495 (1938).
253. K. Kinugawa: Mitt. a. d. med. Akad. zu Kioto *7*: 727 (1933).
254. R. Bierich & K. Kalle: Ztschr. f. physiol. Chem. *175*: 292 (1928).

As far as the amino acid content of cancerous blood is concerned, either no change from the norm [255–257] was found, or only a slight increase (average of 58 cancer sera 4.4 mg. per cent, as compared with a normal average of 3.0 mg. per cent).[258] In rabbits inoculated with Brown-Pearce carcinoma a lowered blood arginine content was observed, and since a similar finding was obtained for pregnant rabbits, this arginine diminution was attributed to growth processes utilizing this substance.[259] In addition, it was reported that a lowering of the blood arginine, which can be produced by the administration of arginase activating substances (ferric chloride, glutathione, cysteine), is much more marked in tumor-bearing animals than in normal rabbits.[260]

Creatinine and guanidine. Creatine and creatinine determinations of mouse and rat tumors furnished values of 30–60 mg. per cent,[261] or of 40–50 mg. per cent, respectively.[188] These values exceed those of other tissues, excepting muscle, heart, and nerve. A slight increase of the guanidine concentration was reported for the blood of cancer patients (average of 2.75 mg. per cent, normal average 2.0 mg. per cent).[262]

The *histamine* concentration in the blood of a few cancer patients investigated was found to correspond to normal values; following irradiation of the tumors, and obviously

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[255] C. H. Greene, K. Sandiford & H. Ross: J. Biol. Chem. *58*: 845 (1923).

[256] S. L. Malowan: Arch. Tierheilk. *65*: 279 (1932); abstr. Ztschr. f. Krebsforsch. *39*: Ref. 20 (1933).

[257] E. Becher & E. Herrmann: Deutsches Arch. f. klin. Med. *173*: 1 (1932).

[258] A. Goldfeder: Ztschr. f. Krebsforsch. *40*: 394 (1934).

[259] M. Reiss, F. Fleischmann & L. Schwarz: Ztschr. f. physiol. Chem. *234*: 201 (1935).

[260] M. Reiss, L. Schwarz & F. Fleischmann: Ztschr. f. physiol. Chem. *236*: 73 (1936).

[261] E. Boyland: J. Physiol. *75*: 136 (1932).

[262] J. Ducuing, F. H. Colombies & J. Lansac: 1st internat. Cancer Congress Madrid, Vol. II, 503 (1933).

in consequence of tissue destruction, the histamine blood values increased.[263]

Nitrogen excretion in the urine. This problem is closely associated with protein metabolism, and the results obtained must be understood in connection with the relations discussed later. No uniform results may be expected, since in different stages of the tumor development the interfering processes of cell proliferation, and of cell decomposition, will be reflected in the urinary excretion of nitrogenous compounds, and the resulting findings will depend on the prevalence of either of these processes. Furthermore, dietary factors and nutritional disturbances, accompanying the disease, will be of decisive importance. Finally, it must be borne in mind that similar phenomena will also be connected with other, nonmalignant conditions of increased growth, or of tissue decomposition. Consideration of these circumstances must govern the interpretation of the following reports. A diminished nitrogen excretion has been observed in patients suffering from uterine cancer, and also in rabbits inoculated with Kato sarcoma.[264] A great many studies have been devoted to the increased excretion of the colloidal nitrogen fraction in the urine of cancer patients.[265-271] It is of no great relevance whether this urine fraction is partially or entirely identical with the so-called oxyproteic acids, as was surmised. For, these substances, which have been defined as nitrogenous and sulfurous, high-molecular, acid oxidation products of proteins have, with justice, been doubted to represent chemically

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263. G. Segal: Compt. rend. Soc. de biol. *131*: 1079 (1939).
264. Y. Fujita: Jap. J. Obst. & Gynec. *19*: 583; 589; 597 (1936).
265. H. Salomon & P. Saxl: Wien. klin. Wchnschr. *22*: 1021 (1909).
266. K. Kojo: Ztschr. f. physiol. Chem. *73*: 416 (1911).
267. E. Salkowski: Berlin. klin. Wchnschr. *47*: 533; 1746 (1910).
268. R. Sassa: Biochem. Ztschr. *64*: 195 (1914).
269. M. Damask: Wien. klin. Wchnschr. *28*: 499 (1915).
270. W. J. Reid: Med. Chronicle *28*: 125 (1913); *29*: 20 (1914).
271. M. Weiss: Biochem. Ztschr. *27*: 175 (1910).

uniform compounds.[272] A subdivision of these substances, performed according to their precipitation by lead and mercury acetate (alloxy-autoxy- and true oxyproteic acids),[273] did not assume any significance in relation to the findings in cancer patients. The methods employed in the studies mentioned consisted mainly of precipitations of the colloidal nitrogen fraction by means of complicated procedures, using in succession barium, lead and mercury salts. Simpler, though less characteristic, measures were also used, such as precipitation by means of ethyl alcohol,[267] determination of the sulfur which can be oxidized by hydrogen peroxide,[274, 275] or determination of the neutral sulfur.[271] These latter fractions, however, were recognized as being mostly identical with thiocyanate, as stated in the chapter on inorganic chemistry. The values obtained for oxyproteic acids, by means of metal salt precipitation, were found to be at least twice the normal in urine of cancer patients.[266] However, these findings were neither constant nor specific for malignant tumors and, correspondingly, their diagnostic utilization has been rejected.[276] A more characteristic nature was ascribed to the increase of the undialysable part of the colloidal urinary nitrogen precipitated by metallic salts.[277] A more recently developed technique for the isolation and determination of the urinary oxyproteic acids showed these substances to represent more than two per cent of the total urinary nitrogen in 76.5 per cent of 102 analyzed cancer patients, whereas it did not exceed 1.5 per cent in normal persons, and patients with sarcoma and

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272. O. v. Fürth: Lehrbuch der physiologischen u. pathologischen Chemie, I: 564.

273. St. Bondzynski & K. Panek: Ber. deutsch. chem. Ges. *35*: 2959 (1903).

274. H. Salomon & P. Saxl: Wien. klin. Wchnschr. *24*: 499 (1911).

275. E. E. Pribram: Wien. klin. Wchnschr. *24*: 1235 (1911).

276. S. Meidner: Ztschr. f. Krebsforsch. *11*: 408 (1912).

277. P. L. J. de Bloeme, S. P. Swart & A. J. L. Terwen: Biochem. Ztschr. *65*: 345 (1914).

other diseases, excepting tuberculosis.[278] These results also suggest a connection with tissue decomposition rather than a primary expression of malignancy.

An increase of the polypeptide nitrogen, excreted in the urine of cancer patients,[279] must be considered in the same light and as reflecting the increased polypeptidemia mentioned. A similar interpretation is due to the urinary excretion of proteoses noted in two thirds of analyzed cancer patients, which finding was also obtained in febrile conditions.[280] An increased excretion of amino acids by tumor patients has been correlated with liver disturbances, especially with metastases.[281, 282]

Proteins and tumor growth. Several attempts have been made to influence the tumor process by modifying the protein administration, be it by withdrawal of certain protein components, or by peroral or parenteral administration of proteins and protein components. Additional work relating to this problem is dealt with in the chapters on nutrition, and on immunology, respectively. As far as the lack of certain amino acids in the ingested protein is concerned, it is in satisfactory agreement with the already mentioned importance of hexone bases for general growth that a diet deficient in these components (lysine, histidine, arginine) inhibited the metastases formation of inoculated tumors.[283, 284] Likewise, the growth of Jensen sarcoma was retarded if lysine was excluded from the diet of the animals.[285] It has been demonstrated in an extensive study that a lysine-poor diet resulted in a strong inhibition of the general growth of young mice, and spontaneous mouse tumors reacted in the same way, if the lysine deficient gliadin formed the only protein source of their diet. After addition of lysine in the form of glutenin, growth was resumed at a normal rate;[286] tryptophane withdrawal from the diet failed to influence the growth,[286] but on the other hand, arginine administration increased the number of takes and enhanced the growth of transplanted mouse tumors.[287]

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[278] B. Lustig & K. Tüchler: Biochem. Ztschr. *289*: 143 (1937).

[279] F. Falk, H. Salomon & P. Saxl: Med. Klin. *5*: 510 (1910).

[280] H. Ury & E. Lilienthal: Arch. f. Verdauungskr. *11*: 72 (1905).

[281] M. Labbé & Mouzaffer: Compt. rend. Soc. de biol. *91*: 1029 (1924).

[282] R. Truhaut & C. Sannié: Bull. Assoc. franç. p. l'étude du cancer *22*: 414 (1933).

[283] J. E. Sweet, E. P. Corson-White & G. J. Saxon: J. Biol. Chem. *15*: 181 (1913).

[284] P. Rous: Bull. Johns Hopkins Hosp. *26*: 146 (1915).

[285] R. Courrier & G. Coste: Compt. rend. Soc. de biol. *115*: 631 (1934).

Special significance has been ascribed to the sulfhydryl compounds present in protein. The development of Flexner-Jobling carcinoma, inoculated to rats, was markedly suppressed by a diet deficient in cysteine.[288] The growth of young mice and of spontaneous mouse tumors was impeded when the animals were supplied with a diet free of cystine; here, too, addition of sulfhydryl compound (cystine, glutathione) caused the growth to resume to a normal extent.[289] While it was thus established that the sulfhydryl groups of the protein represent an indispensable factor for normal and pathologic growth, other authors advanced another step in their theories by assigning to these compounds a decisive influence also on cell differentiation and genetic relations.[290, 291] The product of incomplete oxidation of cysteine, cystine disulfoxide, was found to inhibit the growth of spontaneous mouse tumors significantly.[291] Injection of dl-methionine sulfoxide at first retarded the growth of spontaneous mouse tumors, but later frequently exerted a tumor promoting influence.[292] The injection of cysteine hydrochloride has been reported to cause necrosis and regression of Jensen rat sarcoma.[293, 294] The same author found this procedure effective only if cysteine was applied directly into the tumor, as intraperitoneal injections and peroral administration were followed only by weak results; furthermore, the response of other tumors was still less marked, e.g., intravenous cysteine injections failed to give any results in Brown-Pearce carcinoma.[295] Other authors also saw scarcely any effect from cysteine injections into spontaneous and transplanted mouse tumors.[296, 297]

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[286] C. Voegtlin & M. E. Maver: Pub. Health Rep. *51*: 1429; 1436 (1936).

[287] E. Gilroy: Biochem. J. *24*: 589; 1659 (1930).

[288] A. Goerner & M. M. Goerner: Am. J. Cancer *16*: 360 (1932).

[289] C. Voegtlin, J. M. Johnson & J. W. Thompson: Pub. Health Rep. *51*: 1689 (1936).

[290] F. S. Hammett: Science *83*: 57 (1936).

[291] S. P. Reimann & F. S. Hammett: Am. J. Cancer *26*: 554 (1936).

An interesting phenomenon was observed when l-proline was injected into mice affected with spontaneous tumors. The incidence of multiple tumors was significantly enhanced but, at the same time, the tumor volume diminished, cell differentiation was promoted, and the life of the animals was somewhat prolonged.[298] On the basis of experiments with a marine hydroid, a differentiation promoting property was ascribed to l-proline.[299]

Series of examinations of a large number of amino acids, in regard to their influence on tumors, have been performed independently by several groups of workers. In one of these studies, daily injections of 0.5 c.c. of a one per cent solution of the respective amino acids per 100 gm. body weight of the animals were administered to carcinoma-bearing rats.

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[292] F. S. Hammett: Growth *5*: 85 (1941).

[293] C. L. Connor, J. L. Carr & L. Ginzton: Proc. Soc. Exper. Biol. & Med. *34*: 374 (1936).

[294] J. L. Carr: Proc. Soc. Exper. Biol. & Med. *35*: 341 (1936).

[295] J. L. Carr, C. L. Connor & L. L. Ginzton: Am. J. Cancer *34*: 428 (1938).

[296] E. E. Jones: Proc. Soc. Exper. Biol. & Med. *42*: 403 (1939).

[297] M. J. Eisen & A. Langer: Enzymol. *2*: 321 (1938).

[298] F. S. Hammett: Proc. Soc. Exper. Biol. & Med. *45*: 601 (1940); Growth *5*: 69 (1941).

[299] F. S. Hammett & S. P. Reimann: Ann. Int. Med. *13*: 1483 (1940).

Growth promotion was ascribed to glutamic acid and to arginine, growth inhibition to histidine, leucine and tryptophane, whereas glycine, alanine and phenylalanine were found to be without effect.[300] In another series of experiments, amino acids have been tested, both regarding their action in vitro on cancer cells (carcinolytic test) and with regard to their influence on tumors in vivo, when administered simultaneously with Ehrlich carcinoma transplants.[301] The vitro experiments showed a carcinolytic quality of aspartic acid, phenylalanine, and histidine; a cancer cell protecting quality was seen from glycine, methylglycine, leucine, glutamic acid, ornithine, lysine, arginine, cysteine, cystine, proline and tryptophane. Glycylglycine, alanine, valine, methionine and oxyproline were found inactive. In animal experiments tumor inhibition was obtained with valine, leucine, glutamic acid, ornithine, lysine, arginine, cystine, phenylalanine, proline and oxyproline; methylglycine activated tumor growth and the remaining amino acids were without any influence. As may be seen from this enumeration, the action of the compounds tested in vitro did not always parallel the action in vivo; obviously, in the latter respect the tumor inhibitions observed must be considered merely as expressions of an unspecific toxic effect. Experiments performed with tar cancer and transplanted mouse tumors suggested to other workers a tumor suppressing property of alanine, cystine, aspartic acid and proline.[302,303] It was claimed that mixtures of these amino acids to some extent also benefited cancer patients.[304, 305] The discrepancies, which are obvious with regard to the action attributed by the authors of the mentioned studies to various amino acids,[300-305] are not surprising, in view of the differences apparent with the tested tumors, the dosage, and with the form of application of amino acids. Generally, it may only be deduced that certain protein compounds are indispensable for the tumor growth just as they are for normal growth. Other effects, especially of tumor inhibition, frequently must be correlated with the toxic effects of unphysiologically high concentrations of isolated amino acids.

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[300] K. Suzuki & S. Miyao: Tr. Jap. Path. Soc. *23*: 671 (1933).

[301] B. Lustig & H. Wachtel: Biochem. Ztschr. *278*: 312 (1935).

[302] F. Vlès, A. de Coulon & J. Nicod: Compt. rend. Acad. d. sc. *189*: 1205 (1929).

[303] F. Vlès & A. de Coulon: Arch. physique biol. *11*: 5 (1933).

[304] F. Vlès & A. de Coulon: Bull. Acad. de méd., Paris *110*: 744 (1933).

[305] F. Vlès: 1st Internat. Cancer Congress, Madrid I: 486 (1933).

Polypeptide injections, representing protein derivatives of larger molecular size, were found to increase the growth of rat tumors,[306] obviously by supplying the tumor with needed structural material.

Attempts were repeatedly made to employ decomposition products of tumor proteins for therapeutic purposes, a trend undoubtedly closely related to immunologic conceptions. For such purposes hydrolyzed cancer protein ("ultra-peptones") was recommended,[307, 308] or products of treatment of tumor proteins with high pressure steam ("atmide albumoses") were proposed.[309] Animal experiments, in which tumor-bearing mice were injected with the phosphotungstate precipitate of hydrolyzed tumor proteins,[310] belong to the same category. A rather doubtful rationality underlies a therapy using peroral administration of globulin obtained from homologous tumors.[311] The clinical results reported cannot con-

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306. I. Grundland & H. Bulliard: Compt. rend. Soc. de biol. *131*: 889 (1939).

307. F. Figari: Arch. di biol. *7*: 3 (1930); abstr. Ztschr. f. Krebsforsch. *34*: Ref. 248 (1930).

308. F. Figari & L. Sivori, in Neumann: Streiflichter ueber das Karzinom, 1933, p. 37.

309. R. Bauer & W. Nyiri: Wien. klin. Wchnschr. *34*: 520 (1921).

310. R. Werner & H. Winter: Ztschr. f. Krebsforsch. *39*: 89 (1933).

311. H. Rubens-Duval: Bull. Assoc. franç. p. l'étude du cancer *19*: 154 (1930); *21*: 119 (1931); Progrès méd. *1*: 609 (1931).

vince one of the usefulness of this measure, or of any other of the therapeutic proposals mentioned. This applies also to a "biologic therapy" employing injections of plant proteins isolated from mushrooms and soy beans.[312]

3. *Nucleoproteins and Nucleic Acids.*

These compounds which represent important chemical constituents of the nuclear substance should meet with great interest, in view of the morphologic changes which have been observed in the nuclear and chromatic substance of malignant cells and which have been claimed to characterize the neoplastic process.[313-316] If chemical work has not yet been able to furnish the counterpart to these histologic findings, technical difficulties of isolation and analysis of these substances must be held responsible, obstacles which only the achievements of the last decades seem able to surmount.

Nucleoproteins. According to a report quoted above,[181] an increase of this fraction was found in malignant tumors corresponding to their high nuclear content. A chemical alteration of the tumor nucleoproteins was, however, not detected.[182] A nucleoprotein, prepared from liver metastases of a gastric cancer, exhibited a remarkably high iron content (0.68 per cent),[317] but the isolated character of this finding does not permit any generalization or interpretation. Another investigation attempted a fractionation of nucleoproteins by extracting the tissue, a) with 10 per cent saline, b) with 0.1 per cent sodium hydroxide. Nucleoproteins soluble in the latter medium were found only in considerable quantities in

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312. W. Karo: Ztschr. f. Krebsforsch. *32*: 331 (1930).
313. Th. Boveri: Zur Frage der Entstehung maligner Tumoren, Jena 1914.
314. R. J. Ludford: Brit. M. J. *1*: 68 (1931).
315. M. Levine: Am. J. Cancer *15*: 144 (1931).
316. W. C. McCarty & E. Haumeder: Am. J. Cancer *20*: 403 (1934).
317. A. Oswald: Ztschr. f. physiol. Chem. *97*: 265 (1916).

spleen, kidney and malignant tumors, but the product prepared from neoplasms exhibited no significant chemical differences compared with the nucleoproteins derived from normal organs.[318] Extensive studies have been recently performed on the nucleoprotein fraction of the Jensen rat sarcoma. A great many physical and chemical properties were analyzed and compared with the data of normal nucleoproteins. The substance derived from the tumor proved to be especially rich in sulfhydryl groups, and exhibited a highly amphoteric nature.[319] In continuation of this work, nucleoproteins prepared from transplanted rat hepatomas were compared with nucleoproteins derived from normal rat liver. Although these analyses failed to disclose any significant differences, the authors of the study felt that it would be premature to draw final conclusions from these experiments.[319a] By means of radio-active ("labeled") phosphorus, the nucleoprotein metabolism of liver tumors of rats, produced by butter yellow, has been investigated and, compared with normal rat liver, been found markedly increased.[320] This finding, and similar results obtained with human tumors,[321] may be considered as expressions of the high mitotic activity of neoplasms.

Experimental material on nucleoproteins is scarce and unsatisfactory. Only one report is available, according to which Ehrlich mouse carcinoma has not been consistently influenced by administration of nucleoproteins prepared from normal organs or from tumors. Both inhibition as well as promotion of the tumor growth were occasionally noted, for which the degree of purification of the employed products appeared to be responsible.[322]

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[318] A. Bolaffi: Biochim. e terap. sper. *20*: 133 (1933).

[319] J. P. Greenstein, J. W. Thompson & W. V. Jenrette: J. Nat. Cancer Inst. *1*: 367 (1940).

[319a] J. P. Greenstein, W. V. Jenrette & J. White: J. Nat. Cancer Inst. *2*: 305 (1941).

[320] T. P. Kohman & H. P. Rusch: Proc. Soc. Exper. Biol. & Med. *46*: 403 (1941).

[321] L. A. Erf & J. H. Lawrence: Proc. Soc. Exper. Biol. & Med. *46*: 694 (1941).

Nucleic acids and their components. On account of the technical difficulties mentioned, several workers did not attempt the isolation of pure nucleic acids from neoplasms, but confined themselves to studies of their components, mainly of the purine bases. One author reported he was able to isolate considerable amounts of adenine and hypoxanthine from malignant tumors, whereas only traces of guanine and xanthine could be detected.[323] Conclusions which have been inferred from these findings, as to the enzymatic properties of tumor tissue, will be dealt with in the proper chapter. Quantitative determinations of the total purine-nitrogen showed that this fraction represents a lower percentage of the total nitrogen in malignant tumors than in normal organs. In the same investigation, the guanine content was found slightly to exceed that of adenine in tumors; the quantitative relations of the remaining purines did not differ from the amount in normal tissues.[324] An interesting correlation between nuclear chemistry and vitamins has been revealed by a study, according to which administration of carotene (provitamin A) significantly increased the purine content of normal rat organs and of Jensen rat sarcoma;[325] the relative diminution of the ratio (purine-nitrogen/total nitrogen) was confirmed in work with rat tumors. Nutritional factors, tested in this study, influenced the ratio in question, insofar as a protein-rich diet still further lowered the purine-nitrogen, whereas a vitamin A-free diet failed to affect the purine-nitrogen.[326]

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322. U. Bertolotto: Tumori n.s. *6*: 601 (1932).
323. T. Saiki: J. Biol. Chem. *7*: 23 (1909).
324. H. G. Wells: Proc. Am. Soc. Biol. Chem. VI. s.p. 10 (1912).
325. H. v. Euler & G. Schmidt: Ztschr. f. physiol. Chem. *223*: 215 (1934).
326. S. Edlbacher & P. Jucker: Ztschr. f. physiol. Chem. *240*: 78 (1936).

Sarcoma cells cultivated in vitro were shown by histochemical methods to contain more nucleic acids than normal fibroblasts under the same conditions.[327] Some information regarding the content of nucleic acids and nucleoproteins in tumors may be gained from analyses which determined the distribution of bound phosphorus between nucleic substances and between lipids. These analyses ascertained that, in comparison with normal tissues, malignant tumors exhibited an increased nucleic phosphorus and a diminished lipid-phosphorus.[328-330] All these results may obviously be explained by the increased content of nuclei in malignant tumors.

The few investigations in which a chemical isolation of nucleic acids from tumors and their quantitative analysis have been undertaken did not lead to conclusive results. Some of these experiments appeared to indicate a variation of the nitrogen content of tumor nucleic acid, both if this value is expressed in percentage of the analyzed substance, or if it is correlated with the phosphorus content determined in the same nucleic acid (phosphorus-nitrogen ratio). Whereas thymonucleic acid, prepared from normal organs according to the procedure of Levene,[331] showed a nitrogen content of 14–16 per cent and a phosphorus-nitrogen ratio of 1:1.76, in nucleic acids isolated from malignant tumors (liver metastases) the nitrogen content averaged 9.8 per cent, and a phosphorus-nitrogen ratio of 1:1.2–1:1.0 was obtained.[332, 333] These findings seemed to agree with the results of the studies mentioned above, indicating a decreased purine content [324, 326] and an augmented nucleic phosphorus content [328-330] in malignant tumors. However, other investigations failed to confirm

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327. E. V. Cowdry: Science *68*: 138 (1928).
328. B. Wolter: Biochem. Ztschr. *55*: 260 (1913).
329. Enselme & J. Enselme: Compt. rend. Acad. d. sc. *184*: 1353 (1927).
330. A. H. Roffo & P. Pilone: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *7*: 623 (1930).
331. P. A. Levene & L. W. Bass: Nucleic acids, New York, 1931.
332. R. Willheim: Biochem. Ztschr. *163*: 488 (1925).
333. K. Stern & R. Willheim: Biochem. Ztschr. *272*: 180 (1934).

the diminished nitrogen content of tumor nucleic acids,[334, 335] but the extent of the work done on this problem does not seem to be sufficient to warrant definite conclusions. Qualitative color reactions, testing the carbohydrate content of tumor nucleic acids, revealed no change from the normal thvmonucleic acid.[332, 334]

An augmented concentration of purines and nucleosides, found in the serum of cancer patients, was considered as being dependent on the tissue destruction accompanying the advancing disease.[336] A similar explanation may be assigned to the purine increase noted in the liver of tumor-bearing rats.[326]

Regarding the influence of thymonucleic acids on animal tumors, it was reported that administration of this substance inhibited the growth of transplanted mouse carcinoma [337] and of rabbit tar carcinoma [338] An unspecific mechanism of this action must be considered, in view of the property of nucleic acids of causing leucocytic infiltration and consecutive tissue destruction. Furthermore, another worker found injections of sodium nucleinate to be without effect on transplanted and spontaneous mouse tumors; addition of this substance to tissue cultures caused only a slight growth inhibition.[339] However, an attempt was made, with apparent success, to differentiate normal and tumor nucleic acids in regard to their action on cancer cells. By means of the carcinolytic test, nucleic acids prepared from normal organs were found to exert a lytic action on cancer cells, whereas tumor

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[334] G. Klein & J. Beck: Ztschr. f. Krebsforsch. *42*: 163 (1935).

[335] R. B. Vowles: So. Vet. Ark. Kemi *14B*: No. 101 (1940); abstr. H. v. Euler & I. Petterson: Ztschr. f. Krebsforsch. *51*: 193 (1941).

[336] J. Weber & W. Schuler: Ztschr. f. d. ges. exper. Med. *102*: 45 (1937).

[337] R. Brancati: Tumori *9*: 1 (1922).

[338] J. Maisin: Internat. Conf. Cancer, London, p. 37 (1928).

[339] E. W. Miller: Ztschr. f. Krebsforsch. *45*: 252 (1937).

nucleic acids protected cancer cells against lysis. Yeast nucleic acid was shown to be inactive. Animal experiments yielded analogous results; tumor growth inhibition or prevention by administration of normal nucleic acid; and tumor growth stimulation by nucleic acid as well as by guanylic acid isolated from malignant tumors.[340]

4. *Lipids.*

One appears justified in attributing, for more than one reason, especial importance to this group of biologic substances within the scope of tumor pathology. To begin with, it has been almost universally recognized that the permeability of the cell walls must be viewed as a significant factor for normal and pathologic growth. It has been established beyond all doubt that phospholipids and sterols display a regulatory function in the cell permeability; on the other hand, the work of recent years has revealed a striking chemical relationship existing between certain potent carcinogenic substances and some sterol derivatives. Finally, immunologic phenomena have been demonstrated as being closely connected with lipids and their physiological activities, as will be discussed later. The results which have been accomplished in this field of tumor chemistry and which are dealt with here appear in many instances promising, but must be considered only a starting point for further developments. In any event, it seems that even today no theory of malignant growth, and perhaps also of carcinogenesis, can afford to neglect lipid and sterol chemistry.

Neutral fats. These substances enter the range of the relations outlined above only to a very small extent, because the role of neutral fats, even in normal tissues, generally depends more on their quality of representing a nutritional reserve material than on a participation in the structure and the functional activity of cells. Accordingly, nutritional factors and metabolic activity govern the deposit of these reserves, and pathologic conditions affect them only indirectly. Therefore it is not surprising that neutral fat determinations in malignant tumors have neither evoked great interest, nor have led to identical results in the few instances recorded. One author reported that animal tumors, as well as the body of tumor-bearing animals, showed a reduced content of neutral fat.[341] Undoubtedly these findings are solely indications of the impaired nutritional condition of the animals. Conflicting results have been obtained in another study in which the neutral fat content of some human and animal tumors was found to be increased.[342] For an explanation of these discrepancies, in addition to nutritional factors, the varying fat content of the tumor matrix must also be taken into consideration.

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[340] B. Lustig & H. Wachtel: Ztschr. f. Krebsforsch. *41*: 468 (1935).

A chemical characterization of the fat present in malignant tumors has been attempted by means of the iodine number, although this value reflects the presence of unsaturated fatty acids not only within the neutral fat molecules, but also those contained in phospholipids, or attached to sterols. As compared with an average of 60.2 for the iodine number of normal tissue fat, in sarcomas an increase to 69.8, in carcinomas to 74.4, has been reported; the values for the tumor-surrounding tissue ranged between 65–70.[343] Other determinations of the immediate surroundings of malignant tumors yielded numbers as high as 74.[344] The significance of these findings becomes clear by correlating them with the fact that young tissues generally exhibit a higher concentration of unsaturated fat, and correspondingly higher iodine numbers, than do adult tissues. Hence this phenomenon must be viewed as an indication of the augmented growth rate of malignant tumors. The fact that feeding of unsaturated (olein-rich) fat resulted in an increase of the iodine number of animal tumors[345, 346] may be considered as proof for the direct utilization of ingested fat by the neoplasms.

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[341] A. Bolaffi: Biochim. e terap. sper. *18*: 77 (1931).

[342] M. Yasuda & W. R. Bloor: J. Clin. Investigation *11*: 677 (1932).

[343] A. N. Currie: J. Path. & Bact. *25*: 213 (1922).

[344] Reeves; quot. A. H. Roffo & J. Thomas: La chimie du cancer, Paris, Vigot frères 1933, p. 106.

A certain influence of fat compounds on tumor development has been demonstrated by a number of investigators. As far as the dietary fat composition is concerned, this work will be discussed in the chapter on nutrition. Intraperitoneal injections of olive oil were reported to inhibit the takes of subcutaneously inoculated mouse tumors.[347] It cannot be decided whether this effect was due to the chemical nature of the olive oil, or perhaps rather to its ability for producing a lymphocytosis and, in this way, stimulating the defensive mechanism of the body.[348] An entirely different experimental arrangement was practised by an author who found that the suspension of Ehrlich carcinoma cells in olive oil increased their virulence, as evidenced by their subsequent inoculation to mice.[349] Here similar circumstances seem to exist as in experiments according to which the admixture of tributyrin to the inoculated tumor material also increased the yield of tumors.[350] It has been assumed that this action was due, not so much to the chemical nature of this fat, but to its property of reducing the surface tension which, in turn, would enhance tumor growth (cf. chapter on physical chemistry).

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[345] M. Yasuda: Ronas Ber. *64*: 284 (1932).

[346] B. Kellner & B. Lustig: Biochem. Ztschr. *254*: 214 (1932).

[347] W. Nakahara: J. Exper. Med. *35*: 493 (1922); *38*: 315 (1923).

[348] J. B. Murphy & W. Nakahara: J. Exper. Med. *31*: 1 (1920).

[349] N. Franicevic: Bull. Assoc. franç. p. l'étude du cancer *25*: 733 (1936).

[350] C. Kagan: Ztschr. f. Krebsforsch. *21*: 453 (1924).

A direct tumorigenic action of fatty substances seems possible under special conditions. At least in a few isolated instances, malignant tumors could be observed following the injection of lard and other fats, which have frequently been used as solvents for carcinogenic hydrocarbons,[351] but such an effect doubtless occurs only exceptionally. However, the heating of olive oil or animal fats to 350° C apparently caused the formation of substances of higher carcinogenic potency, since their addition to the diet of rats produced malignant tumors of the stomachs and the intestines in about twenty per cent of the animals treated.[352] Similarly, subcutaneous injections of overheated cotton-seed oil produced sarcomas in two out of twelve mice at the site of the injection, whereas unheated oil failed to induce tumors.[353]

Fatty acids. These substances represent intermediate metabolic products of neutral fats as well as of lipids; they are used also for esterification of sterols and seem, furthermore, to be connected with the intermediate carbohydrate and protein metabolism. Correspondingly, the amount of free fatty acids present in any given tissue will depend on its metabolic and enzymatic activity. A low fatty acid content observed in some malignant tumors [354] can perhaps be interpreted as a result of an increased formation of sterol esters. In carcinomatous lymph glands, a great part of the fatty acids has been identified as oleic acid, whereas the fatty acids present in sarcomatous and tuberculous glands contained a larger amount of double bonds.[355] High concentrations of free fatty acids were found in melanotic liver tumors.[356]

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[351] H. Burrows, I. Hieger & E. L. Kennaway: Am. J. Cancer *16*: 57 (1932); J. Path. & Bact. *43*: 419 (1936).

[352] A. H. Roffo: Bol. inst. med. exper. para el estud. y trat. d. cáncer *15*: 407 (1938); Bull. Assoc. franç. p. l'étude du cancer *28*: 556 (1939).

[353] S. Beck: Brit. J. Exper. Path. *22*: 299 (1941).

[354] A. H. Roffo: Prensa med. Argent. *16*: 493 (1931).

[355] B. Lustig & E. Mandler: Biochem. Ztschr. *263*: 50 (1933).

[356] B. Lustig & E. Mandler: Biochem. Ztschr. *263*: 58 (1933).

The quantity of fatty acids in the blood has been determined with conflicting results, probably depending on differences in the general condition of the tumor hosts. An increased fatty acid content was reported for the blood of rabbits painted with tar from the start of precancerous lesions.[357] High fatty acid values were also claimed to prevail in cancer patients.[358] In women suffering from cancer of the sex organs the fatty acid level of the blood was found to reach the upper limit of the norm; only in very advanced cases, and following roentgen or radium treatment, more marked increases were recorded.[359] On the other hand, the blood serum of Rous sarcoma-bearing fowl was reported to exhibit slightly decreased amounts of fatty acids,[44] and more recent studies of clinical material also showed lowered values for the fatty acids in the blood of cancer patients.[360] Several authors have attempted to prove qualitative changes of the fatty acids present in the blood and organs of tumor hosts; thus it was claimed that the molecular weight of plasma fatty acids was markedly higher in 120 cancer cases than in normal controls.[361] An especial significance has been attributed to the fact that sera of cancer patients contained a large amount of unsaturated fatty acids, which can be extracted by cold ether.[362] This increase of disposable fatty acids was not only employed as a diagnostic aid, but was also considered as an important factor involved in the physical chemistry and the metabolism of malignant tumors (cf. the respective chapters). This view seemed supported by analyses of tumor-free

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357. M. Sendrail: Thèse de Toulouse, 1925.
358. L. Cannavo: Boll. Soc. ital. biol. sper. *2*: *272* (1927).
359. K. Klaus: Biochem. Ztschr. *220*: 204 (1930).
360. R. Reding, N. I. Jonkovsky & A. M. Timmerman: Compt. rend. Soc. de biol. *130*: *273* (1939).
361. Y. Pourbaix & R. Buytaert: Compt. rend. Soc. de biol. *115*: 1408 (1934).
362. M. Ascoli: Klin. Wchnschr. *14*: 1593 (1935).

livers and kidneys of Ehrlich carcinoma mice[363] and of Jensen sarcoma rats.[364, 365] The total amount of unsaturated fatty acids (T) was determined by alcohol-ether Soxhlet extraction, and cold ether extraction yielded the values for the disposable fatty acids (D). The subsequently calculated ratio T/D showed much lower values for the organs of tumor-bearing animals than those obtained from normal controls, thus indicating an increase of the fatty acids soluble in cold ether. It has been confirmed that the quantity of unsaturated fatty acids, extractable by cold ether, is increased in the blood of cancer patients,[366, 367] but the specific nature of this finding, justifying a diagnostic application, has been denied.[368]

A great significance for tumor pathology has been ascribed to certain fatty acids by E. Freund[228] in his theory of resistance and predisposition to cancer. This author and his school assumed that under normal conditions saturated, high-molecular dicarboxylic acids are formed from ingested fat (palmitin, stearin) in the intestinal tract by bacterial action ("normal" bact, coli). These acids, which should then appear in the humoral media, were claimed to be able to dissolve cancer cells, as evidenced by the carcinolytic action of normal serum in vitro, thus protecting the body against cancer formation. Lack of these acids, and the formation of unsaturated dicarboxylic acids by the action of a pathologic intestinal flora, were alleged to be responsible for creating a disposition to cancer. Although these relations, and also controversial statements, will be discussed in detail later, it may be men-

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363. F. Guercio: Tumori n. S. *11*: 1 (1937).
364. R. Indovina & F. Guercio: Biochim. e terap. sper. *23*: 12 (1936).
365. G. Quagliarello: Tumori n.s. *11*: 393 (1937).
366. G. Agnetti: Giorn. clin. med. *17*: 1105 (1936).
367. G. Oliva & G. Fassone: Arch. per le sc. med. *68*: 477 (1939).
368. K. Dirr & E. Sommer: Ztschr. f. d. ges. exper. Med. *107*: 393 (1940).

tioned here that Freund viewed the chemical structure of these fatty acids as directly connected with their opposite action on cancer cells; carcinolytic properties were believed to be exhibited by all fatty acids of greater molecular size of the type $COOH-(C_2H_4)_n-COOH$, whereas cancer cell protecting acids should possess a structure corresponding to maleic acid, i.e., of an unsaturated character. Actually, experiments with tissue cultures showed that the saturated dicarboxylic acids, suberic acid and especially sebacic acid, exerted a strong cytolytic action on carcinoma and sarcoma cells, while fibroblasts were not affected.[369]

No specific chemical, but only a necrotizing effect, appears involved in the local application of butyric acid which has been employed in uterine and rectal cancers.[370]

Oleic acid, an unsaturated monocarboxylic acid, has been studied by several authors with regard to its influence on neoplasms. Intraperitoneal injections of oleic acid dissolved in paraffin, or ferric oleate, inhibited or prevented the formation of tar cancer in mice.[371] This tumor inhibiting action of oleic acid was also apparent in other experiments with tar cancer of mice, the substance being applied externally to the skin of the animals. This effect, however, was explained as being the result of a debilitating effect of this particular fatty acid, and it is well known that animals, whose general health is impaired, are less susceptible to malignant tumors. Furthermore, the tumor inhibiting action was restricted to those experiments in which tar and oleic acid were applied to different parts of the skin, whereas subsequent application of both substances at the same location resulted in an enhanced

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369. M. A. Goldzieher & T. Péterfi: Ztschr. f. Krebsforsch. *31*: 361 (1930).

370. J. Watson: Lancet *1*: 746 (1933).

371. J. Lecloux: Compt. rend. Soc. de biol. *93*: 832 (1925); Cancer, Bruxelles *8*: 37; 59 (1932).

tumor formation.[372] Unsaturated fatty acids, isolated from pig pancreas, and found identical, for the greatest part, with oleic acid, restrained the development of inoculated fowl sarcoma. This effect was markedly reduced by the shortening of the carbon chain, by hydroxyl substitution, and by hydrogenation of the fatty acids. Correspondingly, the saturated stearic acid exhibited a much weaker action; fatty acids with more double bonds (linoleic, linolenic acid) did not show a greater effectiveness than oleic acid. Neutral oils were found active only after saponification.[373, 374]

Total lipids. The total lipid content of malignant tumors has been described as being augmented.[375, 376] A lipid increase of the upper strata of the epidermis was found by histologic methods in skin cancer, but was also seen in seborrhoic acne.[16] Carcinomatous lymph nodes showed a higher lipid content than sarcomatous or tuberculous ones; [354] an account of the different lipid fractions studied in these organs is given later. In the blood of tumor-bearing animals, and also of cancer patients, an increase of the lipid level has been observed; values of up to two per cent were obtained, as compared with 0.3–0.6 per cent normally.[377] This lipid augmentation was accompanied by a reduction of the lipid enzymes, but doubtless such an extreme lipemia occurs in cancer patients only occasionally, due to metabolic disturbances, and does not represent the rule.

Phospholipids. These substances, representing a very important lipid fraction, were found to be increased in animal and human neoplasms.[342, 378-381] The phospholipids appear to be directly connected with the growth process; at least, analysis of the rat carcino-sarcoma 256 showed the growing, non-necrotic, tumor periphery to contain twice the amount of phospholipids than that of the necrotic tumor center.[382] As mentioned in the chapter on inorganic chemistry, this increase of the phospholipids was one of the factors suggesting the use of lead therapy of malignant tumors on the basis that the formation of a lead-lipid compound should deprive the tumors of a vital nutritional material. Investigations which showed a relative decrease of the phosphorus content of lipids isolated from carcinomas and sarcomas,[355, 383] or which showed the lipid phosphorus of malignant tumors to be diminished in comparison with the nuclein phosphorus,[328-330] give no information as to the absolute phospholipid contents of tumors, but describe only the relative concentration to other cell constituents.

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[372] J. M. Twort & C. C. Twort: J. Path. & Bact. *42*: 303 (1936).

[373] O. M. Helmer: J. Exper. Med. *64*: 333 (1936).

[374] O. M. Helmer & G. H. A. Clowes: Am. J. Cancer *30*: 553 (1937).

[375] A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat d. cáncer *5*: 200 (1928).

[376] M. Yasuda: Proc. Soc. Exper. Biol. & Med. *27*: 944 (1931).

[377] H. N. Green & C. N. Jenkinson: Brit. J. Exper. Path. *15*: 1 (1934).

One particular phospholipid, lecithin, has been claimed to reach especially high concentrations in malignant tumors; in dried tumor tissue lecithin values of 5.5–8 per cent were obtained, compared with concentrations of 2.3 per cent in muscle, 4.5 per cent in liver and 7.3 per cent in testis.[384] The high lecithin content, both of tumors and of sex glands, was viewed as being responsible for the increased radiosensitivity of these tissues, by assuming that the radiation finds an important field of attack in the lecithin molecule (cf. paragraph

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378. W. E. Bullock & W. Cramer: Proc. Roy. Soc., London, s.B. *87*: 236 (1913).

379. C. B. Bennett: J. Biol. Chem. *17*: 13 (1914).

380. A. H. Roffo: Bull. Assoc. franç. p. l'étude du cancer *14*: No. 5 (1925).

381. W. B. Bell: Lancet *2*: 1003 (1925).

382. F. L. Haven: Am. J. Cancer *28*: 57 (1937).

383. B. Lustig & E. Mandler: Biochem. Ztschr. *249*: 352 (1932).

384. J. W. Corran & W. C. McC. Lewis: Biochem. J. *18*: 1364 (1924).

on choline, organic chemotherapy). Later studies of rat tumors, however, showed the carcinoma to contain 235 mg. per cent lecithin, and sarcoma 280–360 mg. per cent lecithin, as compared with 151 mg. per cent in normal muscle and 279 mg. per cent in liver.[385] Consequently, at least in these animal tumors, the sarcoma alone seems to exhibit a lecithin increase.

Oleic acid was found to prevail among the unsaturated acids contained in phospholipids, isolated from a transplantable rabbit sarcoma, but only traces of linoleic acid were found.[386] It was reported that a phospholipid containing sulfur, which appeared to be identical with jecorin, could be isolated from animal tumors as well as from human breast cancers.[341, 387]

Phospholipids, it has been recognized, fulfill two different functions in tissues; first, they participate in the structural composition of the cells; second, they may serve for metabolic purposes. By feeding elaidic acid to normal rats, this acid partially replaces the normal acids of the phospholipids, but disappears very rapidly in those organs such as liver, where phospholipids are used functionally. In organs, however, where phospholipids fulfill structural, but not functional tasks, both the appearance and the disappearance of the ingested elaidic acid is much slower. These latter relations were found to prevail in normal muscle as well as in rat tumors, thus indicating that phospholipids are involved in the structure rather than in the function of these tissues.[388] This is in a certain concurrence with the result of studies performed by means of radio-active phosphorus, which revealed the phospholipid metabolism of various transplanted tumors to be largely independent of the host organism, and to be characteristic for the single neoplasm.[389] The turnover of lecithin and cephalin also has been determined by the same method in the rat carcino-sarcoma 256, indicating a more rapid metabolism of lecithin as compared with cephalin. The ratio of lecithin: cephalin was found to be 45:55.[390]

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[385] W. R. Witanowski: Bull. acad. pol. sc. Cl. med. No. 4/6 (1931); abstr. Ztschr. f. Krebsforsch. *36*: 114 (1932).

[386] Y. Sueyoshi & K. Miura: J. Biochem. *29*: 48 (1939).

[387] A. Bolaffi: Biochim. e terap. sper. *18*: 372 (1931).

[388] F. L. Haven: Proc. Am. Soc. Biol. Chem. *13*: Meet., p. 47 (1936); J. Biol. Chem. *118*: 111 (1937).

A compound, aminoethyl phosphoric acid, obviously representing a product of the intermediate phospholipid metabolism, has been isolated in appreciable quantities from malignant tumors, although it is lacking in most of the normal tissues investigated.[391] This finding acquires additional significance in view of the fact that this compound was found to be identical with some natural inhibitors of normal growth.[392]

Choline, a constituent of the lecithin molecule, has been reported as increasing in mouse tumors, parallel with their growth, whereas the body of the tumor-bearing animals showed a progressive impoverishment of this substance. Close to the death of the animals, choline was also found reduced in the tumors.[393]

Phospholipids in blood. On the basis of qualitative tests (precipitation of ether extracts with acetone), a diminution of phospholipids has been supposed to take place in the serum of cancer patients,[394] and more exact analytical methods seem to substantiate this assumption.[395] The phospholipid content in sera of sarcoma-bearing fowl, however, was found unchanged.[258] A qualitative deviation of the lecithin structure has been claimed to occur in the blood of cancer patients, inasmuch as the choline, normally present, was substituted by neurine,[396] but this assumption, based on results of physicochemical measurements, has not been supported by direct analytical evidence.

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[389] H. B. Jones, I. L. Chaikoff & J. H. Lawrence: J. Biol. Chem. *133*: 319 (1940).

[390] F. L. Haven: J. Nat. Cancer Inst. *1*: 205 (1940).

[391] E. L. Outhouse: Biochem. J. *30*: 197 (1936); *31*: 1459 (1937).

[392] A. M. Brues, Y. Subbarow, E. B. Jackson & J. C. Aub: J. Exper. Med. *71*: 423 (1940).

[393] A. Bolaffi: Tumori n. S. *6*: 209 (1932).

[394] E. Freund & G. Kaminer: Biochem. Ztschr. *28*: 312 (1910).

[395] G. de Voss: Ztschr. f. physiol. Chem. *205*: 20 (1932).

Influence of phospholipids on tumor growth. Experimental results seem to bear out a tumor growth-inhibiting action of these substances, in contrast to the growth promoting influence of cholesterol, discussed later. The tumor inhibiting effect which was observed, following parenteral or peroral administration of phospholipids and lecithin in animal experiments [338, 340, 397–399] was, however, less pronounced than the opposite action of cholesterol. Clinically slight improvements, cautiously interpreted by the author, were seen in a few cases of the injection of phospholipids of animal origin.[400] A special affinity for tumor tissue has been ascribed to phospholipids,[401, 402] and their clinical use in combination with peroxides has been recommended.[403] Lecithin injections strongly impeded the growth of fowl and rat sarcoma, whereas rat carcinoma and rabbit sarcoma were influenced only to a lesser degree.[404] An activation of the reticulo-endothelial system was made responsible for this action, which was proved by histologic examinations and by the increase of the number of circulating monocytes.[405, 406]

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396. F. Danmeyer & L. Treplin: Ztschr. f. Krebsforsch. *45*: 171 (1937).
397. S. Bernstein & H. Elias: Ztschr. f. Krebsforsch. *26*: 1 (1928).
398. P. Rondoni: Ztschr. f. Krebsforsch. *32*: 416 (1930); Boll. Soc. ital. biol. sper. *7*: 964 (1932).
399. E. Juster & R. Huerre: Compt. rend. Soc. de biol. *115*: 1489 (1934).
400. H. S. Penn: M. Rec. *142*: 213 (1935).
401. Magat: Ztschr. f. Krebsforsch. *27*: 378 (1928); *39*: 219 (1933).
402. Magat & W. A. Collier: Ztschr. f. Krebsforsch. *39*: 239 (1933).
403. Magat: 1st Internat. Cancer Congress, Madrid II: 1015 (1933).
404. S. Amano & S. Hayashi: Tr. Soc. Path. jap. *25*: 695 (1935); Libro de Oro, Buenos Aires, 1935, p. 5.
405. I. Hayashi & S. Hayashi: Gann *30*: 446 (1936).
406. S. Amano & S. Hayashi: Gann *30*: 449 (1936).

Paralleling the growth inhibition of the tumors, lecithin injections were also followed by an increase of the carcinolytic power of the serum.[407] Since lecithin, added in vitro, did not affect the carcinolysis,[340] this effect in vivo obviously depends on an active participation of the organism. The validity of such a conception is corroborated by a study performed with tissue cultures: plasma obtained from normal fowl, several days after they had received intravenous lecithin injections, inhibited in vitro, the growth of sarcoma cells and of fibroblasts whereas a lecithin addition itself, in vitro, was ineffective.[408] The activity of lecithin, however, may also be increased outside the body, by exposure to ultra-violet radiation. Lecithin preparations, thus treated, strongly inhibited the growth of neoplastic tissue cultures,[409] and were reported as giving beneficial results in the treatment of skin cancer.[410-412] It was believed that the increased activity of irradiated lecithin was caused by the liberation of choline from the lecithin molecule.[409] Therapeutic experiments, using choline itself, will be discussed in the last section of this chapter.

Although cephalin was seen to enhance the growth of fowl sarcoma,[406] it has been recommended for therapeutic use in human malignancy, and beneficial results of this treatment have been claimed, particularly as to the relief of pain.[413, 414]

Lysolecithin, which is derived from lecithin by the splitting

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407. K. Ri: Gann *30*: 409 (1936).
408. K. Tokumitsu: Tr. Soc. Path. jap. *26*: 707 (1936).
409. A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *13*: 1; 407; 493 (1936).
410. E. Juster, F. Caillau & R. Huerre: Bull. soc. franc. dermat. & syphil. *41*: 1904 (1934).
411. E. Juster & R. Huerre: J. de méd., Paris *54*: 849 (1934).
412. J. Rizot: Presse méd. 1935: 1440.
413. K. Pelczar: Atti III. conv. naz. lotta contro il cancro, p. 712 (1934); abstr. Ztschr. f. Krebsforsch. *43*: 251 (1936).
414. Report: Bol. Liga contra el cancer *12*: 210 (1937).

off of one unsaturated fatty acid radical, is deserving of attention because of its marked hemolytic and cytolytic quality; however, no experimental work has been published directly elaborating on the influence of lysolecithin on tumors. Since the conversion of lecithin into lysolecithin is also brought about by the action of snake venom in vivo and in vitro, the supposed beneficial action of snake venoms in neoplastic diseases (cf. chapter on immunology) might be connected with the lysolecithin formation. On the other hand, a promotion of tar cancer development, observed following administration of snake venom, has been believed caused by lysolecithin formation.[415]

Cholesterol. The great significance which several authors found necessary to attribute to this substance is partly based on the results of analytical determinations. It has already been stated in an older report [328] that the cholesterol content of a primary liver cancer was found as being as high as 1.4% of the dried substance. Histologic studies of scirrhous cancers revealed an abundant presence of cholesterol in the interstitial tissue, whereas only small amounts were detectable in the cancer cells themselves.[416] A cholesterol accumulation in various malignant tumors was ascertained in later studies,[417-419] in which average values of three per cent, but also values as high as 9–10 per cent, of dried tissue were occasionally found for the tumor cholesterol. Comparative analyses of benign and malignant tumors agreed in this re-

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415. E. Morelli: Sperimentale *84*: 99 (1930).

416. K. Kawamura: Die Cholesterinsteatose, Fischer, 1914.

417. M. Loeper, Debray & J. Tonnet: Compt. rend. Soc. de biol. *85*: 423 (1921).

418. A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *5*: 144 (1929).

419. A. H. Roffo & L. Correa: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *2*: 44 (1926).

spect, by giving to the former a cholesterol content of 0.3–0.7 per cent, while 1–3 per cent were determined in malignant neoplasms.[420] In six epitheliomas of the skin and also in some precancerous skin lesions cholesterol amounts of 1.25–2.25 per cent were found, while normal skin showed an average value of only 0.43 per cent.[421] Likewise, uterine cancers exhibited a cholesterol concentration essentially superior to that of the normal organ,[422] and cholesterol augmentation has also been demonstrated for the Rous sarcoma.[342, 423, 424] It was, however, stated that this phenomenon accompanied the advancing necrosis of the tumor,[423] and a similar behavior has been reported for the Jensen rat sarcoma and other animal tumors.[382, 425] This could be interpreted to indicate that the cholesterol deposition in tumors might be caused as a consequence of tissue decomposition and would represent a secondary phenomenon of regressive changes, but not a factor primarily involved in the tumor growth. But such a view seems to be justified solely regarding cholesterol esters. These compounds have been found as increased in some animal tumors,[426] whereas other workers [378, 427] reported them as diminished. A satisfactory explanation of these discrepancies was given in a study comparing the cholesterol ester content of Jensen sarcoma and of Flexner-Jobling carcinoma of rats; the slowly growing Jensen sarcoma, therefore containing much necrotic material, exhibited high cholesterol ester concentrations, whereas the more rapidly growing Flexner-Jobling carcinoma failed to show such an increase.[428] On the other hand, the newly formed, non-necrotic parts of Jensen sarcoma transplants exhibited a marked increase of free cholesterol,[429] thus proving the correlation of free cholesterol to the growth process. A similar interpretation may be applied to the observation that the cholesterol content of malignant tumors was found inversely proportional to the duration of the life of the tumor hosts.[430] A cholesterol augmentation of neoplasms has also been indicated by studies relating cholesterol to other lipids, as expressed in the ratio $\frac{\text{Cholesterol}}{\text{Fatty Acids}}$ (lipocytic coefficient),[418, 419] or in the ratio $\frac{\text{cholesterol}}{\text{phospholipids.}}$[426, 431] In both instances, malignant tumors exhibited higher values. Likewise, it has been demonstrated that cholesterol represented a greater percentage of the total lipid content of malignant tumors than of normal organs, as expressed in the ratio $\frac{\text{Cholesterol}}{\text{Total Lipids}}$.[417] Analogous results were obtained by determining the cholesterol content of lipid extracts of organs; 7–20 per cent cholesterol was found in extracts of 13 malignant tumors, whereas normal organs yielded values of 1.2–5 per cent. It is notable that embryonic tissue also showed values of only 1.6–3 per cent.[432] Since in this study the lipids were extracted from isolated tumor cells which had been freed of necrotic material and decomposition products, the results mentioned acquire an especial significance as evidence of a primary connection between cholesterol and malignant growth. In the same light, the findings may be considered that the ratio cholesterol: phospholipids was markedly higher in the peripheral growing parts of the Ehrlich mouse carcinoma than in the central necrotic tumor parts.[433] This view seems also supported by experiments with tissue cultures. Neoplastic cells caused a greater decrease of the cholesterol content of the medium employed than normal ones (80 per cent for tumor cells, 16 per cent for normal ones),[434] and it may be inferred from this observation that malignant cells are utilizing a greater amount of cholesterol. Roentgen or radium irradiation of rat tumors was said to result in a decrease of the cholesterol content,[435] which might imply a reversion to the norm as a consequence of a therapeutic measure.

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[420] F. Burgheim & W. Joel: Klin. Wchnschr. *8*: 828 (1929); *10*: 397 (1931).

[421] C. M Vargas: Urol. & Cutan. Rev. *36*: 310 (1932).

[422] A. de Palo: Riv. ital. di ginec. *22*: 273 (1939).

[423] V. Morávek: Arch. clin. Cancer Res. *4*: 129 (1933).

[424] M. Sumi & W. Nakahara: abstr. Cancer Rev. *6*: 427 (1931).

[425] I. A. Bronstein & D. V. Wolkensohn: Acta Cancrol. *1*: 205 (1935).

[426] R. Bierich, A. Detzel & A. Lang: Ztschr. f. physiol. Chem. *201*: 158 (1931).

[427] A. Bolaffi: Ztschr. f. Krebsforsch. *30*: 170 (1930).

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[428]. R. Bierich & A. Lang: Ztschr. f. physiol. Chem. *216*: 217 (1933).

[429]. A. Lang & A. Rosenbohm: Ztschr. f. Krebsforsch. *48*: 183 (1938).

[430]. R. Bierich & A. Lang: Klin. Wchnschr. *15*: No. 19 (1936).

[431]. A. Lang: Ztschr. f. Krebsforsch. *49*: 20 (1939).

[432]. R. Willheim & G. Fuchs: Biochem. Ztschr. *274*: 279 (1932).
(1932).

Cholesterol in fat deposits, and in organs of the tumor hosts. A cholesterol increase in the liver of tumor animals has been indicated by a high lipocytic coefficient, i.e., a cholesterol augmentation in relation to the fatty acids, whereas the fat deposits of the tumor animals did not show such a behavior.[436] But determinations of the unsaponifiable fraction of the fat deposits of cancer patients yielded higher values than those shown by corresponding analyses of normal persons,[437] thus making a cholesterol increase probable. However, since similar findings were obtained in tuberculosis, chronic sepsis and diabetes, the theory was offered that this cholesterol accumulation may represent a defensive mechanism of the body against the acidity of free fatty acids by binding them to cholesterol. In support of such a view, attention was drawn to the fact that in the conditions enumerated,

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433. F. M. Brikker & Y. A. Lazaris: Ztschr. f. Krebsforsch. *37*: 432

434. A. H. Roffo: Bol. inst. de med. exper. para el estud. y. trat. d. cáncer *9*: 503 (1932).

435. A. H. Roffo & L. Correa: Ztschr. f. Krebsforsch. *22*: 79 (1925).

436. L. Correa: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *10*: 511 (1933).

437. L. Wacker: Ztschr. f. physiol. Chem. *78*: 349; 383 (1912).

the adrenals are depleted of cholesterol which normally is stored in them in large quantities. Histologic analyses of the kidneys of Brown-Pearce carcinoma rabbits showed a lipid decrease, and adrenals, kidneys and spleen of guinea pigs, inoculated with a liposarcoma, were found to be cholesterol-poorer than the organs of normal animals.[438] A raised cholesterol content of the bile of cancer patients has been mentioned,[439] and the whole body of tumor-bearing mice has been reported as showing an increased content of cholesterol esters,[427] whereas the spleen of tumor rats was found to accumulate cholesterol, parallel to the advancing tumor growth.[440] Increased cholesterol concentrations were also observed in lung, liver and spleen of sarcoma-bearing fowl.[441] While the reports mentioned do not bear a uniform character, the conclusion seems justified that the tumor process is accompanied by a far reaching deviation of the cholesterol distribution in the organism, probably depending on the specific nature and the stage of the neoplasms.

Light and cholesterol. In view of the facts enumerated, and particularly in consideration of the experimental material discussed later, especial significance has been attributed to the phenomenon that the cholesterol content of normal rat skin could be markedly increased by exposure to sunlight, or, to a slighter degree, by exposure to ultra-violet rays.[442] This subject was extensively elaborated by Roffo and his collaborators.[443, 444] These authors established a direct relation between the cholesterol content of the skin

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438. F. Garofalo: Bull. d. sc. med. Bologna *104*: 373 (1932); abstr. Am. J. Cancer *21*: 667 (1934).

439. S. J. S. Peirce: Deutsches Arch. f. klin. Med. *106*: 337 (1912).

440. A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *10*: 59 (1933); Ztschr. f. Krebsforsch. *41*: 44 (1934).

441. T. Takizawa: Gann *31*: 125; 370 (1937).

442. S. Kawaguchi: Biochem. Ztschr. *221*: 232 (1930).

443. A. H. Roffo: Presse méd. *1*: 282 (1931); 1st Internat. Cancer Congress, Madrid *2*: 421 (1933); Strahlentherapie *53*: 317 (1935).

444. L. Correa: 1st Internat. Cancer Congress, Madrid *2*: 149 (1933).

and its exposure to sunlight, as evidenced by the following values for various skin parts: abdomen 0.4 per cent, face 0.7 per cent, precancerous skin 1.6 per cent, skin cancer 1.7 per cent These relations were envisaged as explaining the prevalence of skin cancer in parts exposed to intensive solar radiation, which has been statistically ascertained and, in particular, they were connected with experimental tumor production by ultraviolet rays (cf. chapter on physical chemistry). The cholesterol increase in the skin of rats, following ultraviolet radiation, has been corroborated; the response was strongest in the face, weaker in the abdomen and the back of the animal. Tumors, developing subsequently, proved to be rich in cholesterol, eighty per cent of which was of the free form.[445] It was claimed that a previous splenectomy or epinephrectomy prevented the cholesterol increase by ultraviolet rays,[446] perhaps by depriving the organism of its cholesterol reserves. It must be mentioned, however, that other workers contested the connection claimed between the cholesterol content of the skin and the tumor development following ultraviolet radiation.[447]

Blood cholesterol. Statements referring to the blood cholesterol content of cancer patients are highly conflicting. Reports claiming an increase[58, 198, 448–451] are contrasted by others indicating a decrease.[452–454] In addition, some

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445. A. Knudson, S. Sturges & W. R. Bryan: J. Biol. Chem. *128*: 721 (1939).

446. A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *14*: 19 (1937).

447. C. A. Baumann & H. P. Rusch: Am. J. Cancer *35*: 213 (1939).

448. N. Louros & E. Gaessler: Ztschr. f. Krebsforsch. *28*: 191 (1927).

449. W. L. Mattick & K. W. Buchwald: J. Cancer Res. *13*: 157 (1929).

450. L. Bugnard, F. H. Colombies, Miletzky & Boursiac: Bull. Assoc. franç. p. l'étude du cancer *20*: 596 (1931).

451. O. Kingreen: Arch. f. klin. Chir. *177*: 383 (1933).

452. H. Guthmann: Arch. f. Gynäk. *140*: 202 (1930).

453. K. Klaus: Biochem. Ztschr. *220*: 204 (1930).

454. L. von Noël: Strahlentherapie *42*: 609 (1931).

authors[455, 456] arrived at the conclusion that neoplasms did not cause any appreciable change of the cholesteremia. In fourteen early stages of malignant tumors normal blood cholesterol values were obtained, except in one case with a severe anemia in which this disturbance, and not the tumor, was made responsible for a decreased cholesterol content.[455] Likewise, another author found normal values in thirty-one cancer patients.[457] Some workers[458, 459] reported that in the blood of cancer patients the total cholesterol content was not characteristically changed, but that the free cholesterol was increased at the expense of cholesterol esters, shifting the normal ratio of $\frac{\text{Free cholesterol}}{\text{Cholesterol esters}}$ of 1:2 in cancer blood to 1:1–1:1.7.[458] These divergencies might be caused by several factors. To begin with, the use of different substrates (blood, plasma, serum) for the determination may be of importance, according to the findings of one author[48] who observed an unspecific oscillation of the cholesterol content in cancer patients, if whole blood was used, but who also found a marked hypercholesteremia when employing the blood plasma. Later on, however, the same author[459] pointed to an important source of error, inherent in all investigations which had used the colorimetric cholesterol determination method (Liebermann's reaction). By using the more precise measurement of cholesterol by means of the digitonine precipitation, he found the free and total plasma cholesterol in cancer patients to exhibit varying but, on the whole, rather diminished concentrations. A greater difference between the two values was found in cancer blood than in normal blood by simultaneously performing the colorimetric and the digitonine methods. He assumed that the first determination also encompassed other substances (such as oxy-cholesterol), but the increase of this cholesterol-like fraction was not considered as specific for malignancy, since it was also encountered in other processes involving protein decomposition.[459] Furthermore, it was stated that the blood cholesterol values of cancer-free patients of different age and of different pathologies exhibit variations too great to permit an evaluation of the values obtained in cancer patients, although generally lowered concentrations were observed in the last stages of this disease.[460] The results obtained in animal experimentation are likewise not less equivocal. No typical changes of the serum cholesterol have been found in rabbits with tar carcinoma[461] or with Brown-Pearce carcinoma,[59] or in rats with Jensen sarcoma.[462] The observation of a greatly varying cholesteremia in sarcoma-bearing rats[463] may serve as an explanation for divergent reports which claim a hypercholesteremia of tumor-bearing rats[464] and rabbits.[465] In rats inoculated with the Ehrlich-Putnoky carcinoma, a hypercholesteremia developed (220–260 mg. per cent, as compared with a normal average of 155 mg. per cent) in the course of advancing tumor growth; only 10–15 per cent of the total cholesterol was in the form of esters, whereas this fraction normally represented forty per cent, but this deviation was attributed to non-specific factors, i.e., to a hepato-renal damage caused by the resorption of tumor decomposition products.[466]

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[455] **W. Denis**: J. Biol. Chem. *29*: 93 (1917).

[456] **H. R. Downes & G. T. Pack**: Am. J. Cancer *16*: 290 (1932).

[457] **K. Fuge**: Strahlentherapie *50*: 157 (1934).

[458] **R. Harms**: Giessen, Diss. 1935; abstr. Ztschr. f. Krebsforsch. *45*: Ref. 253 (1937).

[459] **R. Reding**: Compt. rend. Soc. de biol. *131*: 843 (1939).

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460. O. Bokelmann & O. Mühlbock: Klin. Wchnschr. *16*: 854 (1937).
461. Y. Yoshioka: Okayama-Igakkai-Zasshi *39*: 167 (1927).
462. C. Kaufmann & R. Erdmann: Biochem. Ztschr. *249*: 438 (1932).
463. F. Dannenberg: Biochem. Ztschr. *244*: 128 (1932).
464. H. N. Green & C. N. Jenkinson: Brit. J. Exper. Path. *15*: 1 (1934).
465. A. R. Harnes: J. Exper. Med. *50*: 109 (1929).
466. S. Sümegi: Frankfurt. Ztschr. f. Path. *48*: 386 (1933).

Blood cholesterol after roentgen irradiation. Since roentgen application generally results in a decreased cholesteremia, it seemed noteworthy that several investigators reported they had found a rise of the blood cholesterol following the irradiation of malignant tumors.[443, 467–469] However, such a behavior was indicated to occur only in non-cachectic cancer patients, and was occasionally also found in cancer-free persons.[470] Furthermore, other authors even observed a decreased cholesteremia in irradiated cancer patients,[471, 472] or denied any regular response of the blood cholesterol to roentgen radiation of tumors.[473, 474] Similarly, no regular reaction seems to be caused by the ultraviolet radiation of the skin, as far as the blood cholesterol content is concerned. The claim that cancer patients, in contrast to cancer-free persons, respond to such a treatment with a decreased cholesteremia [475, 476] has met with a re-examination yielding contrary results.[477]

Further evidence for a disturbed cholesterol distribution in the organism of persons affected with tumors was furnished by the finding that the cerebrospinal fluid, normally containing only traces of this substance, showed a marked cholesterol increase in malignancy.[478]

The cholesterol excretion was found to be increased (more

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467. E. Jacobs & R. Motojima: Ztschr. f. Krebsforsch. *26*: 57 (1928).
468. E. Jacobs: Ztschr. f. Krebsforsch. *32*: 248 (1930).
469. M. Levy-Dorn & F. Burgheim: Strahlentherapie *22*: 538 (1926).
470. V. Jura: Ztschr. f. Krebsforsch. *39*: 374 (1933).
471. R. Hubert: Klin. Wchnschr. *7*: 208 (1928).
472. M. Benetato-Modval & L. Pop: Compt. rend. Soc. de biol. *116*: 1100 (1934).
473. P. H. Schumacher & Rusch: Strahlentherapie *57*: 532 (1936).
474. I. A. B. Cathie: J. Path. & Bact. *43*: 587 (1936).
475. S. Malcsyński: Klin. Wchnschr. *9*: 936 (1930).
476. R. Hubert: Strahlentherapie *44*: 379 (1932); Arch. f. Gynäk. *146*: 51 (1931).
477. H. Schwalm: Arch. f. Gynäk. *152*: 415 (1933).
478. A. H. Roffo: Prensa med. Argent. *13*: 1114 (1927).

than 0.05 mg. per 100 c.c.) in the urine of nineteen out of ninety-two cancer patients, but this behavior was declared as not being characteristic for cancer, since it was found more frequently in kidney diseases, and also in pregnancy. The course of cholesteroluria of cancer patients proceeded parallel with the development of cachexia.[479, 480]

Cholesterol and tumor development. An influence of cholesterol on tumor growth was ascertained as long ago as 1913 by a series of experiments demonstrating the accelerated growth of transplanted tumors following cholesterol injections.[481] Likewise, peroral administration of cholesterol, not in excessive amounts, caused a growth stimulation of rat sarcomas and of mouse carcinomas,[482] and in agreement with this, it was stated that tumor formation by tar was enhanced if accompanied by cholesterol administration.[483–485] Cholesterol-rich diet,[396] as well as cholesterol injections,[397, 443, 444, 486] have been noted as favoring tumor growth. The fundamental difference existing between spontaneous and experimental or transplanted tumors seems evidenced by a report, according to which cholesterol-rich diet failed to influence the incidence of spontaneous tumors in mice.[487] No unanimous opinion prevails as to the influence of cholesterol on tumor production by means of ultraviolet rays. According to the work of Roffo mentioned previously, cholesterol seems essentially involved in the formation of these malignant

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[479] E. Bloch & H. Sobotka: J. Biol. Chem. *124*: 567 (1938).

[480] H. Sobotka, E. Bloch & A. B. Rosenbloom: Am. J. Cancer *38*: 253 (1940).

[481] T. B. Robertson & T. C. Burnett: J. Exper. Med. *17*: 344 (1913).

[482] G. Tesauro: Ztschr. f. Krebsforsch. *35*: 269 (1932).

[483] M. Borst: Ztschr. f. Krebsforsch. *24*: 337 (1924).

[484] R. Jaffe & A. Eliassow: Zentralbl. f. allg. Path. u. path. Anat. *40*: 229 (1927).

[485] W. Eber, F. Klinge & L. Wacker: Ztschr. f. Krebsforsch. *22*: 359 (1925).

[486] K. Wolkoff: Ztschr. f. Krebsforsch. *31*: 291 (1930).

[487] T. B. Robertson & L. A. Ray: J. Biol. Chem. *37*: 443 (1919).

tumors, and this author found his view supported by the fact that ultraviolet irradiation activated cholesterol in vitro, by conferring a photodynamic activity to this substance.[488] This phenomenon was shown, in another investigation, to be caused by ozonide formation and by subsequent liberation of hydrogen peroxide.[489] Actually, subcutaneous cholesterol injections were seen to enhance and to accelerate the formation of malignant tumors in mice exposed to ultraviolet rays,[490] but in another study a cholesterol-rich diet failed to stimulate the tumor production in mice by means of ultraviolet treatment. Likewise, such a diet did not influence hepatoma development in mice fed aminoazotoluene. These results made a tumor-promoting property of cholesterol appear doubtful to the authors.[491]

From a different point of view, a significance of cholesterol for malignant growth has been deduced from vitro experiments. It was noted that cholesterol addition to tissue cultures containing epithelial cells and fibroblasts resulted in a stimulation of the epithelial elements, suppressing and displacing the growth of the fibroblasts. From this observation it was inferred that cholesterol may disturb the balance between epithelial and connective tissues, and thus participate in creating conditions prevalent in malignant epithelial neoplasms.[492] Another author [493] assumed, on the basis of indirect evidence, that the factor which is responsible for the cancer-cell-protecting ("anti-lytic") activity of blood serum

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488. A. H. Roffo & A. E. Roffo: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *12*: 300; 569 (1935).

489. W. V. Mayneord & E. M. F. Roe: Am. J. Cancer *31*: 476 (1937).

490. B. Polettini: Tumori n. s. *13*: 122 (1939).

491. C. A. Baumann, H. P. Rusch, B. E. Kline & H. P. Jacobi: Am. J. Cancer *38*: 76 (1940).

492. M. Katzenstein & E. Knake: Ztschr. f. Krebsforsch. *33*: 378 (1931).

493. A. v. Christiani: Ztschr. f. Krebsforsch. *42*: 25; 317 (1935); *43*: 272 (1936).

of cancer patients, as exhibited in the carcinolytic test, was identical with a cholesterol ester, i.e., cholesterol-n-butyrate. The discussion and evaluation of this theory and of counterclaims will be found in the section on the carcinolytic test.

The question, as to whether cholesterol possesses a tumor-promoting or even a tumor-producing capacity, assumed increased importance in view of the brilliant work of the last years relating to carcinogenic polycyclic hydrocarbons. In the course of these studies the close chemical relationship of some of these compounds to sterols has been revealed, since a common nucleus for these substances, and also for some of the sex hormones, is furnished by the phenanthrene structure. A direct carcinogenic quality is obviously not possessed by cholesterol. The fact that intraperitoneal cholesterol injections into rabbits, to the ear of which tar was applied at the same time, caused the formation of a carcinoma in an inguinal lymph node (metastasis?) in one single animal[494] can scarcely be interpreted in this sense. But the question arose as to whether cholesterol may undergo structural changes inside or outside the body which would imbue it with a carcinogenic property. While no method has as yet been devised to bring about, or to prove such a conversion of cholesterol in the living organism, it has nevertheless been claimed that certain chemical or physical measures may be successfully employed in vitro, which endow cholesterol with a carcinogenic property. However, it cannot be decided at present whether such alterations of cholesterol might have any biologic significance. In this respect, very energetic chemical measures were first employed by subjecting cholesterol to a temperature of 800° C. The resulting pyrogenic products exhibited a definite tumorigenic potency, since painting of mice with these substances resulted in eleven

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494. J. Cholewa: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *14*: 77 (1937).

malignant tumors among the seventy animals treated.[495] By a similar process of exposure to high temperature and destillation, phenanthrene derivatives have been obtained from cholesterol, which proved carcinogenic for rats.[496] While these very deep-reaching chemical changes may scarcely be expected to find a counterpart in biological processes, other changes of a lesser degree were also carried out in vitro which imparted a similar activity to cholesterol. Roentgen irradiated cholesterol, dissolved in fat and injected into mice, produced spindle cell sarcomas in two out of twenty animals after 390 and 544 days, respectively.[497] External application of a similar product to the skin of mice, however, failed to produce any malignant neoplasms.[498] In other experiments cholesterol which previously had been exposed to ultraviolet light, or to sunlight, was admixed with the food of rats, and subsequently ulcers and adenocarcinomatous tumors of the stomach, and also spindle cell sarcomas of the liver, were seen in some of the animals.[499] It must be admitted that these experiments are far from conclusive, and they certainly do not warrant any assumption for ascribing a carcinogenic quality to sterols contained in foodstuffs in their natural form.

Bile and bile acids. The investigations dealing with these substances have mostly been conducted from a viewpoint which has been dominated by the fact that the bile acids, the main components of bile besides cholesterol, are on the basis of their structure closely related to certain carcinogenic compounds, particularly to the highly active methylcho-

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495. E. L. Kennaway & B. Sampson: J. Path. & Bact. *31*: 609 (1928).
496. A. H. Roffo: Ztschr. f. Krebsforsch. *49*: 341 (1939).
497. H. Burrows & W. V. Mayneord: Am. J. Cancer *31*: 484 (1937).
498. W. Bergmann, H. E. Stavely, L. C. Strong & G. M. Smith: Am. J. Cancer *38*: 81 (1940).
499. A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *14*: 589; 657; 665 (1938); Ztschr. f. Krebsforsch. *47*: 473 (1938).

lanthrene.[500] A theory assuming the possible formation of carcinogenic compounds from cholesterol by the action of bile acids [501] must be considered as purely hypothetical for the present. In this connection, some significance may be seen in the observation that bile production was increased in rats which had been inoculated with dibenzanthracene tumors.[502] No increase of the bile acids was found in the blood of forty-nine cancer patients, some of them even affected with liver metastases,[503] a finding which is noteworthy because of the relationship of bile acids to the carcinogenic agents mentioned.

Older experiments in which the administration of bile acids appeared to inhibit the tar cancer formation,[504] or in which the admixture of bile acids with inoculated cancer cells caused necrosis and subsequent failure of tumor development,[340] cannot be considered as evidence of a true tumor-inhibiting property of these substances,[505] but are merely expressions of their toxic and tissue destroying effect. On the other hand, definite proof has been given of a tumor-promoting and even tumor-producing ability of bile acids. In the latter case, experiments have been recorded in which injection of deoxycholic acid resulted in the development of sarcomas in rats and mice,[506] findings which have already been confirmed.[507] Several studies revealed a stimulation of tumor development due to the administration of bile and its components. The

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500. J. W. Cook: J. Chem. Soc. London *1930*: 1087.

501. R. Estripeaut: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *14*: 159 (1937).

502. L. D. Parsons: Nature, London *144*: 75 (1939).

503. D. Abragam: Compt. rend. Soc. de biol. *121*: 950 (1936).

504. V. E. Mertens: Ztschr. f. Krebsforsch. *27*: 295 (1928).

505. M. J. Shear: Am. J. Cancer *25*: 66 (1935).

506. V. Ghiron: Boll. Acad. med. Roma *64*: 134 (1938); 3rd Internat. Cancer Congress Atlantic City, Sept. 1939.

507. J. W. Cook, E. L. Kennaway, N. M. Kennaway: Nature, London *145*: 627 (1940).

growth of a transplanted rat sarcoma (Fujinawa) was enhanced if the animals received injections of sodium desoxycholate,[508] and addition of bile to filtrates of rat sarcomas, produced by dibenzanthracene, increased the percentage of positive inoculation results.[502] Injections of 10 per cent ox bile were given to a mouse strain which showed a 100 per cent incidence of breast cancer in the females, and an incidence of spontaneous hepatomas in 32 per cent of the males. The treatment markedly increased the hepatoma incidence in the males, whereas the tumor development in the females, already maximal, was not influenced.[509] Likewise, injection of sodium desoxycholate significantly increased the incidence of lung cancer in a pure-bred mouse strain.[510]

Miscellaneous lipids. Other lipids, such as sphingomyelin and cerebrosides, have been found in some malignant tumors.[511] A recent investigation established sphingomyelin concentrations of 0.3 per cent of the wet weight of rat carcinosarcoma 256; it was also made probable that sphingomyelin is as important for the lipid metabolism of tumors as is lecithin and cephalin.[512] Histologic examinations of neoplasms indicated an increased content of myelin [513] which, however, is not a chemically defined compound, but a degeneration product. Accordingly, its accumulation may be taken as a sign of regressive changes.

The physical properties may justify the inclusion of the lipochromes with the lipids. These natural dyestuffs, which are related to carotinoids, were reported as being increased in malignant tumors, but this finding has been explained as being due to secondary factors, such as augmented chromosynthesis, or bacterial action, and therefore is not specific for malignancy.[514]

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[508] T. Kondo: Gann *31*: 259 (1937).

[509] F. C. Turner: Pub. Health Rep. *54*: 1603 (1939).

[510] L. W. Law: Proc. Soc. Exper. Biol. & Med. *47*: 37 (1941).

[511] H. Thierfelder & E. Klenk: Die Chemie der Cerebroside und Phosphatide, Springer, Berlin 1930.

[512] F. L. Haven & S. R. Levy: J. Biol. Chem. *141*: 417 (1941).

[513] I. Haga: Berlin. klin. Wchnschr. *49*: 342 (1912).

In conclusion, some studies may be referred to, in which lipids of not chemically defined nature have been investigated as to their influence on the tumor process. These substances represented various mixtures, extracted by suitable lipid solvents, and accordingly varying as to their composition and their action. On the one hand, it was reported that peroral and parenteral administration of lipid extracts, derived from various organs of cattle, promoted the growth of animal tumors;[486] on the other hand, injection of brain lipids were seen to inhibit the tumor development.[515-517] A detailed discussion of the part played by the lipids of the food in connection with the tumor process will be given in the pertinent chapter.

B. *Organic Carcinogenic Substances.*

This section has to deal with a topic which has been elaborated in the most intensive way, especially during the last decade, and correspondingly comprises a vast amount of experimental material, of laboratory and clinical findings, and of theoretical attempts to correlate and to utilize them for an advanced understanding of the cancer problem. There are clearly three different tributaries discernible, the confluence of which finally resulted in the broad stream of present day research on carcinogenic compounds. As the oldest one, clinical observations must be viewed which go back as far as the 18th century, and by which the attention of perspicacious physicians was called to some forms of malignant tumors de-

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514. A. N. Currie: Brit. J. Exper. Path. *5*: 293 (1924).
515. H. Kretzschmar: Ztschr. f. Krebsforsch. *28*: 154 (1928).
516. A. R. Harnes: J. Lab. & Clin. Med. *20*: 1077 (1935).
517. H. Vassiliadis: Compt. rend. Soc. de biol. *118*: 483 (1935).

veloping almost exclusively, and with notable frequency, in persons occupationally exposed to certain organic substances. The progress of industrialization during the nineteenth century furnished more and more pertinent material and, simultaneously, intensified the practical interest in this health hazard, the elimination of which appeared imperative and therefore prompted a great deal of work, which was partly successful in the field of industrial hygiene. A new stimulation for work on this subject has been received from experimental laboratory work, at the time when this branch of cancer research has come into its own and assumed an equal standing by the side of clinical and of pathological studies. Transplanted animal tumors have been mainly used as the necessary experimental material since the beginning of this century when highly efficient procedures were developed, making them freely available. Spontaneous animal tumors have been only infrequently employed on account of the incomplete knowledge prevailing until recently, as to the conditions of making them accessible in the larger quantities required for precise experimentation. Because of these circumstances, and in view of the grave doubts arising as to whether the tumors transmitted for many generations from one animal to another could serve as adequate material for studies directed towards problems of human tumor pathology, it became desirable to an even greater degree to devise some means of experimental tumor production in animals, which would more closely resemble relations of human neoplasia. A promising point of attack for a solution of this problem seemed to be given by the clinical experiences of occupational cancer, obviously caused by organic chemicals, and long lasting efforts in this direction finally met with success in the discovery of Yamagiwa and Itchikawa[518] who, in 1915, announced they had caused the development of true cancerous growth in rabbits by applying coal tar to the ear of the animals for a long period of time. This discovery opened a new era in experimental cancer research, leading to an ever-increasing number of findings, and the disclosure of new problems. Last, but not least, purely chemical work must be credited with solidifying the basis, and enlarging the horizon, of the problem under discussion. The studies concerned were initiated in the early thirties by the efforts of Kennaway, Hieger and Cook and their collaborators, with the object of isolating as pure chemical compounds the effective agents of organic carcinogenic substrates. The means by which the authors succeeded in this endeavor and how the continuation of their brilliant work advanced to the point of synthetic preparation of a great number of tumorigenic organic compounds will be described in the course of this presentation. An attempt will also be made to evaluate the significance of these discoveries and to visualize their theoretical and practical consequences as far as the ample experimental material seems to permit.

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[518] K. Yamagiwa & K. Itchikawa: Mitt. a. d. med. Fak. Univ. Tokyo *15*: 295 (1915); *17*: 19 (1917); *19*: 483 (1918); J. Cancer Res. *3*: 1 (1918); Virchows Arch. f. path. Anat. *233*: 235 (1921).

By far the greatest contingent and the most important representatives of organic carcinogenic substances belong to the polycyclic series. Nevertheless, in accordance with chemical custom, substances of the aliphatic series, which have been shown to be endowed with a tumor-producing property, will be discussed first.

Carbon. This basic element of organic chemistry is deserving of primary consideration because it seems to represent the oldest recognized cause of an occupational tumor development, i.e., the scrotal cancer of chimney sweepers. This disease was first described by the English physician Percival Pott[519] in 1775, and has since been repeatedly studied,[520] especially in England where the greatest amount of clinical material seemed to be available. Although it was obvious that the coal soot with which the chimney sweepers are forced to come into intimate contact must represent an important factor in the development of this particular and otherwise infrequent cancer form, the localization on the scrotum pointed to the participation of additional factors, such as intertriginous friction, pressure of clothes, and intensive perspiration. Further details as to the clinical picture, and as to preventive measures of social hygiene, may be found in special monographs.[521] Perhaps because of improved general hygiene (more frequent change of clothing, more frequent baths), or on account of changed working conditions, even leading to an obsolescence of the profession named, the incidence of chimney sweepers' scrotal cancer has rapidly decreased during the last decades, as indicated by mortality statistics; whereas in 1880 a figure of twenty per cent was given,[522] in the years 1900–1902 only 1.33 per cent were reported,[523] and a statistical analysis published in 1937 furnished similar evidence.[524] The formation of malignant tumors has been occasionally observed also in other workmen exposed to a steady coating of coal and soot particles. It must, however, be carefully considered as to whether the pure carbon element can be held responsible for these phenomena, or whether instead additional organic substances, either contained in the coal, or products of partial combustion (empyreumatic substances), were the real bearers of carcinogenic property. This restrictive interpretation must also be applied to animal experiments which were undertaken with substances derived from coal and, in some instances, led to positive results.

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[519] Percival Pott: The Chirurgical Works, Vol. 3, p. 225, London 1783.

[520] Th. B. Curling: The Diseases of the Testis, Philadelphia 1843.

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[521] K. Ullmann: Krebsbildung in der Gewerbemedizin, Hdb. d. Haut- u. Geschlechtskrankheiten, Jadassohn, Vol. 12, pt. III, pp. 551–720 (1930).

[522] Butler, quot. Ullmann loc. cit. 521.

[523] T. M. Legge: Brit. M. J. *2*: 1110 (1922).

[524] S. A. Henry: Am. J. Cancer *31*: 28 (1937).

Cancers have been produced in mice by means of ether extracts of soot,[525] or by externally applying printers' ink.[526] In other experiments, however, soot application to the scrotum of rabbits, rats or mice failed to elicit tumor formation.[527] As far as internal tumor development is concerned, it has been reported that mice exposed to inhalation of coal soot for a long period of time showed a lung cancer incidence of eight per cent, as compared with two per cent in control animals of the same strain. The authors pointed to a possible analogy in human pathology, insofar as the lung cancer frequency has been found definitely higher in city dwellers, exposed to more smoke inhalation, than in country people, although the establishing of definite conclusions does not seem warranted as yet.[528] Lung tumors have also been induced in mice by means of benzene extracts of chimney soot, and subcutaneous injections resulted either in local sarcoma formation or in primary lung tumors;[529] it is obvious that ether extracts[525] or benzene extracts[529] of soot contain a tarry material, and that therefore these findings must be associated with the category of tar cancer.

Paraffins. In spite of the fact that certain industrial lubricants, containing these aliphatic hydrocarbons, have for a long time been known to cause skin cancer quite frequently in persons handling them,[521] further studies demonstrated that chemically pure paraffins are not endowed with tumorigenic qualities,[527, 530, 531] but that admixed compounds of polycyclic nature were the causative factors for tumor formation (cf. petroleum cancer). This inactivity of the aliphatic

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525. R. D. Passey: Brit. M. J. *2*: 1112 (1922).
526. Steinbrück: Berlin. tierärztl. Wchnschr. *45*: 525 (1929).
527. A. Leitch: Brit. M. J. *2*: 1047 (1922).
528. M. G. Seelig & E. L. Benignus: Am. J. Cancer *28*: 96 (1936).
529. M. B. Shimkin & J. Leiter: J. Nat. Cancer Inst. *1*: 241 (1940).
530. F. C. Wood: J. Cancer Res. *13*: 97 (1929).
531. E. Tantini: Tumori n. s. *9*: 266 (1935).

hydrocarbons is by no means surprising, in view of their general chemical and biological inertness, but it is worthy of emphasis, so as to avoid any undue suspicions regarding their most common use as laxatives and for other medical purposes.

It was recently announced that a high percentage of hepatomas arose in mice when the animals were fed *carbon tetrachloride* for eight to sixteen weeks. It cannot as yet be decided whether this effect indicated a true carcinogenicity of carbon tetrachloride, or whether it resulted merely from a non-specific liver damage.[532]

Ethyl alcohol. It has been reported that prolonged peroral or rectal administration of alcohol to mice resulted in formation of cancers of the stomach, or rectum, respectively,[533] but this effect was considered as the consequence of a chronic irritation only, caused by highly toxic alcohol concentrations, and not as the expression of a direct carcinogenic action.[534] Such a view, rejecting generalizing conclusions drawn from the animal experiments mentioned, is upheld by the complete lack of clinical evidence pointing to alcohol, one of the most widely used beverages, as being etiologically connected with human neoplasia.

Formaldehyde, in the form of formalin-oil emulsions, was found to cause an atypical epithelial growth on the ear or in the stomach of rabbits,[535] but only more extensive investigations can decide whether this simple organic substance is actually capable of producing true malignant tumors.

Lactic acid. It has been mentioned above [121-128] that plant tumors could be evoked by the application of lactic acid, and it

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532. J. E. Edwards: J. Nat. Cancer Inst. *2*: 197 (1941).

533. C. Krebs: Am. J. Obst. & Gynec. *1923*: *67*; Ztschr. f. Immunitätsforsch. u. exper. Therap. *59*: 201 (1928).

534. J. Maisin: 1st Internat. Cancer Congress, Madrid, sec. social, p. 95 (1933).

535. W. G. Garschin: Ztschr. f. Krebsforsch. *45*: 62 (1937).

must be stressed again that one is not justified in correlating these findings in any way with the pathology of animal or human tumors. In this connection, it may be recalled, because of a historical interest, that by means of zinc precipitation a substance was isolated from malignant tumors which was allegedly able to produce malignant neoplasms in animals.[536] This substance was later identified as zinc lactate, but its injection into mice and rabbits has been proven as causing only the formation of unspecific granulomas.[537]

Polycyclic series. One of the most simple compounds belonging in this category is phenol (carbolic acid). A few isolated clinical observations are recorded describing the development of skin cancer following handling of crude carbolic acid.[538] In another instance a mixed epithelioma of the back arose after an ointment containing phenol and ergot was applied over a period of twenty years.[539]

Petroleum cancer. This instance of occupational neoplasia due to the action of a natural organic substrate was described in 1875 under the name of paraffin cancer.[540] But since the investigations mentioned above [527, 530, 531] clearly indicated that chemically pure paraffins are not carcinogenic, the nomenclature petroleum cancer appears more appropriate.[521] Aromatic, phenol-like substances were believed to impart the tumor producing quality to the petroleum and, hence, to be responsible for malignant tumors developing in workmen employed in oil drilling, refineries and similar industries. "Mule-spinners' cancer" is especially well known, a skin tumor very frequent in English spinneries where a lubricant derived from petroleum has been used. Statistical data the modern incidence of this occupational disease were co piled in 1937 [524] and in a very recent study attention v drawn to the continued incidence of Mule-spinners' canc Ichthyosis of the skin, friction of cloth and lack of cleanlin were recognized as predisposing factors, and protective me ures have been discussed.[540a] It has been recognized that pathogenicity of various petroleum varieties is not unifor It was found very high in those of Siam, Sumatra and oth of far eastern origin, whereas Russian and Rumanian pr ucts exhibited a very low carcinogenic potency, and oils fr Pennsylvania and California none at all. Further stud showed that the carcinogenic property is mainly contair in the fractions distilling between 300 and 400° C.[521] It likely that these fractions contain compounds similar to th obtained from tar (see later), and therefore no significa can be ascribed to former assumptions of a carcinogenic tion of arsenic,[541] or of organic sulfur compounds, such mercaptan,[542] admixed with the petroleum.

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[536] H. W. Nowell: Zentralbl. f. allg. Path. u. path. Anat. *24*: No. 15 (1913).

[537] E. Fränkel & W. Klein: Ztschr. f. Krebsforsch. *15*: 76 (1915).

[538] Story, quot. K. Ullmann loc. cit. 521.

[539] J. B. Stevens & J. L. Callaway: Am. J. Cancer *38*: 364 (1940).

[540] Volkmann (1875), quot. O. Teutschländer: Monatschr. f. Krebsbekämpf. *1*: 30 (1933).

Whereas some animal experiments were unable to achie a tumor production by painting with, or by injection petroleum,[543] other workers observed the development papillomas turning into cancerous growth,[544] and maligna animal tumors have been produced in a comparatively hi percentage of cases by means of petroleum fractions of high carcinogenic potency.[527]

Tar cancer. This form of malignancy appears closely lated to the petroleum cancer, in view of the analogy existi between the respective causative agents, since both represe products of dry distillation. Tar is derived from coal an various organic substances, whereas the formation of petr leum most probably resulted from a similar natural proce by which in earlier geological periods vegetable and anim matter were converted into the present substrate. The know edge of industrial tar cancer occurrence preceded by man decades the discovery of the experimental tar cancer. Nume ous experiences demonstrated the considerable hazard of ski cancer formation in workmen handling tarry materials, as i gasworks, coke-ovens, briquette and pitchplants, etc. Int mate contact with tar (as necessitated by the cleaning of co distillation apparatus) and exposure of body parts to ta vapors and pitch dust have been noticed, after a period c incubation frequently lasting many years, to first cause th appearance of skin decolorization and of pigmented warts i some of the workers, followed by inflammatory reaction and finally cancer formation. Certain technical improv ments, installed in the respective plants, which prevent th conversion into dust of the tarry material, together with mor careful individual prophylaxis, have considerably lessene this grave health hazard for workers in the tar industry.[5 In particular, a treatment of the tar and the tarry oils wit sulfur dioxide has been recommended for the purpose o destroying the carcinogenic activity[545] and, without doub further planned work in the field of industrial hygiene ma lead to a complete eradication of this occupational neoplasia In this connection, it seems somewhat ironical that the worl of modern cancer research has created a new group of en dangered persons, that is the experimenters themselves, wh handle such substances for a prolonged period of time in thei laboratory work. At least one report has been recorded o a research worker who, following prolonged animal experi mentation involving application of tar, observed the occur derived from petroleum has been used. Statistical data on the modern incidence of this occupational disease were compiled in 1937[524] and in a very recent study attention was drawn to the continued incidence of Mule-spinners' cancer. Ichthyosis of the skin, friction of cloth and lack of cleanliness were recognized as predisposing factors, and protective measures have been discussed.[540a] It has been recognized that the pathogenicity of various petroleum varieties is not uniform. It was found very high in those of Siam, Sumatra and others of far eastern origin, whereas Russian and Rumanian products exhibited a very low carcinogenic potency, and oils from Pennsylvania and California none at all. Further studies showed that the carcinogenic property is mainly contained in the fractions distilling between 300 and 400° C.[521] It is likely that these fractions contain compounds similar to those obtained from tar (see later), and therefore no significance can be ascribed to former assumptions of a carcinogenic action of arsenic,[541] or of organic sulfur compounds, such as mercaptan,[542] admixed with the petroleum.

[540a] E. M. Brockbank: Brit. M. J. *1*: 622 (1941).

[541] A. Bayet: Cancer, Bruxelles *1*: 5 (1923).

[542] Stallybras, quot. K. Ullmann loc. cit. 521.

[543] K. Ullmann: Wien. klin. Wchnschr. *38*: 38 (1925).

[544] E. Hoffmann, H. T. Schreus & E. Zurhelle: Deutsche m Wchnschr. *49*: 633 (1923).

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[545] C. C. Twort & J. M. Twort: Lancet *1*: 286 (1934).

Whereas some animal experiments were unable to achieve a tumor production by painting with, or by injection of, petroleum,[543] other workers observed the development of papillomas turning into cancerous growth,[544] and malignant animal tumors have been produced in a comparatively high percentage of cases by means of petroleum fractions of higher carcinogenic potency.[527]

Tar cancer. This form of malignancy appears closely related to the petroleum cancer, in view of the analogy existing between the respective causative agents, since both represent products of dry distillation. Tar is derived from coal and various organic substances, whereas the formation of petroleum most probably resulted from a similar natural process by which in earlier geological periods vegetable and animal matter were converted into the present substrate. The knowledge of industrial tar cancer occurrence preceded by many decades the discovery of the experimental tar cancer. Numerous experiences demonstrated the considerable hazard of skin cancer formation in workmen handling tarry materials, as in gasworks, coke-ovens, briquette and pitchplants, etc. Intimate contact with tar (as necessitated by the cleaning of coal distillation apparatus) and exposure of body parts to tar vapors and pitch dust have been noticed, after a period of incubation frequently lasting many years, to first cause the appearance of skin decolorization and of pigmented warts in some of the workers, followed by inflammatory reactions, and finally cancer formation. Certain technical improvements, installed in the respective plants, which prevent the conversion into dust of the tarry material, together with more careful individual prophylaxis, have considerably lessened this grave health hazard for workers in the tar industry.[521] In particular, a treatment of the tar and the tarry oils with sulfur dioxide has been recommended for the purpose of destroying the carcinogenic activity[545] and, without doubt, further planned work in the field of industrial hygiene may lead to a complete eradication of this occupational neoplasia. In this connection, it seems somewhat ironical that the work of modern cancer research has created a new group of endangered persons, that is the experimenters themselves, who handle such substances for a prolonged period of time in their laboratory work. At least one report has been recorded of a research worker who, following prolonged animal experimentation involving application of tar, observed the occur-

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[540a] E. M. Brockbank: Brit. M. J. *1*: 622 (1941).

[541] A. Bayet: Cancer, Bruxelles *1*: 5 (1923).

[542] Stallybras, quot. K. Ullmann loc. cit. 521.

[543] K. Ullmann: Wien. klin. Wchnschr. *38*: 38 (1925).

[544] E. Hoffmann, H. T. Schreus & E. Zurhelle: Deutsche med. Wchnschr. *49*: 633 (1923).

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545. C. C. Twort & J. M. Twort: Lancet *1*: 286 (1934).

rence of multiple papillomas tending to malignant degeneration on his hands.[546] Caution in this respect must therefore be urged, and this naturally holds good to a still greater degree for the handling of the more potent synthetic carcinogenic compounds. In view of the extensive use of tar preparations for external medicinal purposes, it is also noteworthy that cases have been observed in which such a treatment of patients resulted in tar melanosis, keratosis and cancer development.[547]

Although it is not intended to minimize the value of the work reported dealing with the various aspects of occupational tar cancer, its significance is unquestionably far surpassed by the discovery of the experimental tar cancer, credit for which must be given to the tireless efforts of Yamagiwa and Itchikawa,[518] who succeeded where several predecessors had failed. These authors periodically applied tar to the ears of rabbits and, after having continued this treatment for several months, they observed the formation of papillomas, which subsequently were transformed into carcinomatous growths bearing all the characteristics of true neoplasms. Shortly thereafter (1918), analogous results were achieved by tar application to the skin of the back of white mice.[548] These discoveries opened a fertile new branch of experimental cancer research which was taken up by a great number of investigators. Mainly employing mice and rabbits as experimental animals, these studies have generally confirmed the results of the first authors, although certain divergencies became apparent as to the percentage yield of malignant tumors, and as to the average length of time necessary for their development in the animals treated. Differences in the

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546. Anonymous: Ztschr. f. Krebsforsch. *26*: 360 (1928).
547. A. Matras: Dermat. Wschnschr. *1935*: 43.
548. H. Tsutsui: Gann *12*: 17 (1918).

animal strains used, on the one hand, and in the tar material employed on the other hand, may be responsible for these deviations. But it has definitely been established that, provided suitable animals and a proper tar preparation were chosen, positive results could be achieved in the surviving animals after three to six months. In this connection, it soon became evident that the tar treatment evoked a general toxic effect, probably independently of its carcinogenic potency, and caused the death of some of the animals prior to the development of neoplasms; the toxicity was characteristic for the individual tar preparation and depended also on the dosage. Principally, the following three factors have been recognized as determining the tumor formation by means of tar application: one, use of a susceptible animal species proving to be responsive to tumor formation; two, employing of a tar preparation of adequate carcinogenic potency; three, sufficient length of time for the response to develop, including a "latent" and a "preparatory" period.[549] In addition to the painting of the skin, other forms of tar administration have also been successfully tried, and thus malignant tumors have been produced in internal organs. Here it is of interest to refer to studies of more recent date which proved that dust from tarred roads was carcinogenic for mice, producing lung tumors when the animals inhaled this material for a longer period of time,[550] and also producing skin tumors following local application.[551] The wide scope of the studies on tar cancer may be inferred from the fact that a monograph on this subject, published in 1926, even then included a bibliography of 290 papers,[552] and the intensified interest of the sub-

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549. W. H. Woglom: Am. J. M. Sc. *181*: 157 (1931).
550. J. A. Campbell: Brit. J. Exper. Path. *17*: 146 (1936).
551. J. A. Campbell: Brit. J. Exper. Path. *20*: 122 (1939).
552. W. H. Woglom: Arch. Path. & Lab. Med. *2*: 533; 708 (1926).

sequent years has been reflected by a later summary which collected 390 additional papers.[553] The material available may be grouped into several categories, according to the particular purpose for which the respective studies were undertaken. The first group has been devoted to morphological viewpoints, i.e., the analysis of histological changes leading to, and characterizing the tar carcinoma, both locally and in the organs of the affected animals; being outside of the scope of this survey, this material is not dealt with here. The second group of investigations used the production of the tar cancer in animals as a means to an end, that is, for obtaining in a comparatively easy and simple way animal tumors more closely resembling human neoplasms than the transplanted tumors heretofore used. Correspondingly, tar tumors have been employed for all the fields of cancer research detailed in this book, for analytical determinations in inorganic, organic and physical chemistry, for studies of enzymes, for nutritional factors, for metabolism and hormonal interrelationship and for the evaluation of all kinds of therapeutic and of diagnostic measures. Reference to this work is given in the proper chapters, but it will be emphasized here that, under equal conditions, results established with tar tumors (or other chemically induced neoplasms) may frequently possess a greater significance for the understanding of human tumor pathology than those obtained from experimentation with transplanted tumors. Insofar as attempts have been made to draw general conclusions regarding carcinogenesis on the basis of the established factors governing tar cancer formation, these will be discussed later in connection with the chemically pure carcinogenic compounds. This leads to the third group of studies, which attempted to attack the problem in question from the chemical angle, aiming at the isolation of the effective carcinogenic agents in the form of pure and structurally defined substances, a trend which in due course met with remarkable success.

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[553] M. G. Seelig & Z. K. Cooper: Am. J. Cancer *17*: 589 (1933).

The first stepping-stone in this direction was given by the observation that the greatest part of the carcinogenic activity of coal tar was contained in the fractions boiling at higher temperatures.[554] Further important information was gained by comparison of blast-furnace tar and gasworks tar, of which only the second exhibits a strong carcinogenic activity in workmen and in animal experiments. It has been ascertained that the blast-furnace tar contained a greater amount of paraffins, known to be noncarcinogenic, but was deficient in some aromatic components occurring in gasworks tar. In the latter the carcinogenic activity was found to be present in the higher boiling fractions, distilling over at temperatures ranging from 250 to 500° C. None of the known chemical constituents of these fractions could be demonstrated as tumor-producing in animal experiments.[555] Although in later experiments [556, 557] tumor production was achieved in mice by means of blast-furnace tar also, it has been confirmed that the carcinogenic potency of this tar was inferior to that of gasworks tar, and therefore the significance of the aforementioned findings does not seem to be impaired. Attempts were made to determine the concentration of active factors in various tar products and mineral oils by measuring certain chemical and physical properties, so as to avoid the expensive and time-consuming animal experiments which serve the same purpose; encouraging results have been obtained in this respect.[558, 559] But another series of studies has been of a far greater consequence, as is described in the following section. These studies eventually led to a precise insight into the chemical nature of the carcinogenic factors contained in tar and, in addition, initiated the synthetic preparation of a large number of new and powerful carcinogenic compounds.

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[554] B. Bloch & W. Dreifuss: Schweiz. med. Wchnschr. *51*: 1035 (1921).

[555] E. L. Kennaway: Brit. M. J. *1*: 564 (1924).

[556] I. Berenblum: Lancet *219*: 1344 (1930).

[557] G. M. Bonser: Lancet *223*: 775 (1932).

[558] C. C. Twort & J. D. Fulton: Ztschr. f. Krebsforsch. *35*: 543 (1932); J. Hyg. *33*: 464 (1933).

[559] C. C. Twort: Libro de Oro, Buenos Aires, p. 1412 (1935).

In imitation of the industrial tar production, organic material, such as yeast and human skin, but also the simpler organic compounds acetylene and isoprene, were subjected to temperatures extending from 700 to 920° C, and the resulting products were all found to be capable of tumor production in animals.[560] But another chemical procedure, involving a much lower reaction temperature, was also found to lead to organic compounds of carcinogenic potency, viz., Schroeter's synthesis, using aluminum chloride. If tetraline was exposed to the action of aluminum chloride at 30 to 40° C, high-boiling aromatic compounds were obtained which induced malignant neoplasms in suitable animal experiments. At the same time the very significant observation has been made that these aromatic compounds with carcinogenic quality, synthesized from tetraline, and, also, tars and other carcinogenic organic substances all exhibited a very characteristic fluorescence spectrum, with well defined bands at wave lengths of 4000, 4180 and 4400 Ångstrom. A great number of known pure hydrocarbons were then tested as to their spectral qualities, and the spectrum of 1, 2-benzanthracene was found to come nearest to that of the substances mentioned above. The synthesis of several derivatives of 1, 2-benzanthracene yielded products of considerable carcinogenic potency, foremost among them 1, 2, 5, 6-dibenzanthracene and, to a lesser degree, 1, 2, 7, 8-dibenzanthracene.[561, 562] The high carcinogenicity of 1, 2, 5, 6-dibenzanthracene has been confirmed in extensive studies,[563-565] and several other benzanthracene derivatives were likewise found capable of producing malignant neoplasms, such as 6, 7-dimethyl-1, 2-benzanthracene, 6-methyl-1, 2-benzanthracene, and 5, 6-cyclopentenon-1, 2-benzanthracene.[566] Although a new class of potent carcinogenic compounds of known chemical structure, condensed ring aromatic hydrocarbons, was thus detected, the primary purpose of identifying the chemical agent responsible for the carcinogenicity of coal tar and similar substrates did not seem accomplished, since benzanthracene or its derivatives were not found to occur among them. But utilizing the phenomenon of the characteristic fluorescence spectrum inherent to carcinogenic agents, a concentration of the active fraction of coal tar was performed [567] and, by employing suitable chemical methods, from this fraction a pure compound of extremely great carcinogenic potency was isolated and identified as 3, 4-benzpyrene.[568, 569] Although it is by no means established or claimed that this polycyclic hydrocarbon represents the only active agent of coal tar, this accomplishment must be viewed as a remarkable piece of work, particularly when it is recalled that only nine years earlier one of the authors of this group,[555] after having searched in vain among the known constituents of tar for

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[560] E. L. Kennaway: J. Path. & Bact. *27*: 233 (1924); Brit. M. J. *2*: 1 (1925).

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561. E. L. Kennaway: Brit. M. J. *1*: 1044 (1930); Biochem. J. *24*: 497 (1930).

562. I. Hieger: Biochem. J. *24*: 505 (1930).

563. H. Burrows: Proc. Roy. Soc. London s. B *111*: 238 (1932).

564. P. R. Peacock: 1st Internat. Cancer Congress Madrid *1*: 80 (1933).

565. G. Barry & J. W. Cook: Am. J. Cancer *20*: 58 (1934).

566. P. R. Peacock: J. Path. & Bact. *36*: 141 (1933).

567. I. Hieger: J. Chem. Soc. London 1933: 395.

568. J. W. Cook, C. L. Hewett & I. Hieger: J. Chem. Soc. London 1933: 396.

569. J. W. Cook: 1st Internat. Cancer Congress Madrid *2*: 373 (1933).

the carcinogenic agent, compared this effort with the attempts to isolate and identify vitamins or hormones from a complex biologic substrate. In addition to the benzanthracene derivatives and benzpyrene, a third group of aromatic hydrocarbons, exhibiting a powerful activity of tumor production, is represented by cholanthrene and, especially, by 20-methylcholanthrene. The latter substance has been independently synthesized by two groups of authors,[570, 571] and its great carcinogenicity has been corroborated by several investigators.[572-575]

The availability of these carcinogenic compounds brought about extensive experimentation which confirmed the original statements. Tumor production by means of 1, 2, 5, 6-dibenzanthracene was accomplished in rabbits,[576] in mice,[575, 577-582] in rats,[583, 584] and in fowl.[585] The carcinogenic activity of benzpyrene was found greatest in mice, least in rabbits,[585, 586] and this compound or its derivatives were tested in several

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570. J. W. Cook & G. A. D. Haslewood: Chem. & Indust. *38*: 758 (1933).

571. H. Wieland & E. Dane: Ztschr. f. physiol Chem. *219*: 240 (1933).

572. G. Barry, J. W. Cook, G. A. D. Haslewood, C. L. Hewett, I. Hieger & E. L. Kennaway: Proc. Roy. Soc. London s. B *117*: 318 (1935).

573. A. C. Bottomley & C. C. Twort: Am. J. Cancer *21*: 781 (1934).

574. J. W. Cook: Berichte *69A*: 46 (1936).

575. M. J. Shear: Am. J. Cancer *26*: 322 (1936).

576. A. Lacassagne: Compt. rend. Soc. de biol. *114*: 660 (1933).

577. J. Maisin & P. Liégois: Compt. rend. Soc. de biol. *114*: 536 (1933).

578. M. G. Seelig: Am. J. Cancer *20*: 827 (1934).

579. L. M. Schabad: Ztschr. f. Krebsforsch. *42*: 295 (1935).

580. H. B. Andervont: Pub. Health Rep. *50*: 211 (1935).

581. N. Dobrovolskaia-Zavadskaia: Compt. rend. Soc. de biol. *121*: 1268 (1936).

582. T. W. Lettinga: Thesis 1937, abstr. Am. J. Cancer *31*: 495 (1937).

583. J. Maisin & M. L. Coolen: Compt. rend. Soc. de biol. *117*: 109 (1934).

584. E. Boyland & H. Burrows: J. Path. & Bact. *41*: 231 (1935).

585. I. Berenblum & L. P. Kendal: Brit. J. Exper. Path. *15*: 366 (1934).

586. C. Sannié, C. Oberling, M. Guérin & P. Guérin: Compt. rend. Soc. de biol. *120*: 1196 (1935).

other studies.[587-591] Although in some experiments benzpyrene failed to elicit tumors in rabbits,[592] positive results were obtained by other workers;[593, 594] nevertheless this animal species seems to be less susceptible to the stimulus of this carcinogenic hydrocarbon. Likewise, the carcinogenic effect of methylcholanthrene has been investigated extensively,[595-601a] and why especial biological significance has been attributed to this compound and its derivatives will be discussed later.

It must be emphasized here that this survey is by no means intended to give a complete and detailed account of the subject in question, but it is merely an attempt to retrace the most important steps of the pertinent investigations, and to outline the results derived from them, particularly as far as conclusions of general significance for the tumor problem appear warranted. Excellent reviews summarizing the re-

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587. C. Oberling, C. Sannié, M. Guérin & P. Guérin: Bull. Assoc. franç. p. l'étude du cancer *25*: 156 (1936).

588. P. Rondoni & A. Corbellini: Atti med. naz. Lincei VI s. *21*: 128 (1935).

589. O. Schürch & A. Winterstein: Ztschr. f. physiol. Chem. *236*: 79 (1935).

590. G. Domagk: Ztschr. f. Krebsforsch. *44*: 160 (1936).

591. M. J. Shear: Am. J. Cancer *36*: 211 (1939).

592. O. Lambret, J. Driessens & M. Cornillot: Compt. rend. Soc. de biol. *124*: 61 (1937).

593. J. Klinke: Ztschr. f. Krebsforsch. *46*: 334 (1937).

594. O. Schürch: Ztschr. f. Krebsforsch. *49*: 353 (1939).

595. H. B. Andervont: Pub. Health Rep. *53*: 1647 (1938).

596. H. L. Stewart: Am. J. Path. *15*: 707 (1939).

597. J. L. Nicod & J. Regamey: Bull. Assoc. franç. p. l'étude du cancer *27*: 706 (1938).

598. P. Valade: Bull. Assoc. franç. p. l'étude du cancer *27*: 723 (1938).

599. J. Maisin & M. L. Coolen: Compt. rend. Soc. de biol. *123*: 159 (1936).

600. L. F. Fieser, M. Fieser, E. B. Hershberg, M. S. Newman, A. M. Seligman & M. J. Shear: Am. J. Cancer *29*: 260 (1937).

601. W. F. Dunning, M. R. Curtis & M. J. Eisen: Am. J. Cancer *40*: 85 (1940).

601a. L. W. Law & M. Lewisohn: Cancer Research *1*: 695 (1941).

ported work on carcinogenic substances have been published,[602-607] among which especial attention is directed to the presentations of Cook, Kennaway and their collaborators [604, 605, 607] who, themselves, participated in the elaboration of this subject. It may be taken as an indication of the tremendous scope which the pertinent studies have reached that this review which collected the material up to 1938 included a bibliography of 860 papers. A survey recently published [607a] covers all compounds tested for carcinogenicity and reported up to and through 1939. This work is particularly valuable for the reason that it not only includes 696 inorganic and organic compounds studied, but it also assembles all available data pertaining to the scope, arrangement and results of the experiments. It therefore renders possible a correct interpretation of the experimental results and of the conclusions drawn from them, and it points to supplementary or corroborating work where needed.

The carcinogenic hydrocarbons have been applied either externally by periodically painting the skin of the animals with oily or benzene solutions, which procedure mostly resulted in carcinomatous growth, or subcutaneous, intramuscular or intraperitoneal injections of the hydrocarbons, dissolved in lard, oil or cholesterol pellets, have been administered, in one single or in repeated injections, and, in these experiments tumors of sarcomatous or carcinomatous nature

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602. J. W. Cook, G. A. D. Haslewood, C. L. Hewett, I. Hieger, E. L. Kennaway & W. V. Mayneord: 2nd Internat. Cancer Congress, Bruxelles, Vol. *1*: 1 (1936).

603. R. Kinosita: Tr. Soc. path. jap. *27*: 665 (1937).

604. J. W. Cook, G. A. D. Haslewood, C. L. Hewett, I. Hieger, E. L. Kennaway & W. V. Mayneord: Am. J. Cancer *29*: 219 (1937).

605. J. W. Cook & E. L. Kennaway: Am. J. Cancer *33*: 50 (1938).

606. C. Dittmar: Protoplasma *32*: 598 (1939).

607. J. W. Cook & E. L. Kennaway: Am. J. Cancer *39*: 381; 521 (1940).

607a. J. L. Hartwell: Survey of Compounds which have been tested for carcinogenic activity, Federal Security Agency, U. S. Pub. Health Service, 1941.

resulted, depending on the tissue which underwent the neoplastic transformation. Water-soluble colloidal compounds have also been prepared from the carcinogenic substances mentioned and successfully used for tumor production.[588, 608–613] Various internal tumors have been observed following suitable administration of the carcinogenic compounds: feeding of benzpyrene dissolved to 0.4 per cent in fat, caused gastric cancers, forming metastases, in five out of six mice, after 112 to 336 days;[614] kidney tumors have been produced in mice by means of minute amounts of 1, 2, 5, 6-dibenzanthracene,[615] and prostate tumors—carcinomas and sarcomas—in white rats by injections of benzpyrene.[616] Oral ingestion of dibenzanthracene or methylcholanthrene in mice resulted in the formation of adenocarcinomas of the small intestine, or the formation of lung tumors,[617] but other authors assigned only a weak carcinogenic effect to the oral administration of methylcholanthrene.[618] Bone tumors of malignant nature have also been induced in mice by means of methylcholanthrene and benzpyrene.[619, 620] Recently injections of methylcholanthrene were employed for inducing adenocarcinomas in the stomach of mice.[620a] Implantation of methylcholanthrene pellets into the brains of mice resulted in

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608. I. Berenblum: Lancet *2*: 1107 (1932).
609. E. Boyland: Lancet *2*: 1108 (1932).
610. A. Winterstein & H. Vetter: Ztschr. f. physiol. Chem. *230*: 169 (1934).
611. L. F. Fieser & M. S. Newman: J. Am. Chem. Soc. *57*: 1602 (1934).
612. H. Burrows & J. W. Cook: Am. J. Cancer *27*: 267 (1936).
613. P. H. O'Hara & J. A. Pollia: Am. J. Cancer *31*: 493 (1937).
614. N. Waterman: Acta brev. Neerland *7*: No. 1 (1937).
615. F. W. Ilfeld: Am. J. Cancer *26*: 743 (1936).
616. R. A. Moore & R. H. Melchioma: Am. J. Cancer *30*: 731 (1937).
617. E. Lorenz & H. L. Stewart: J. Nat. Cancer Inst. *1*: 17 (1940).
618. J. Van Prohaska, A. Brunschwig & H. Wilson: Arch. Surg. *38*: 328 (1939).
619. A. Brunschwig & A. D. Bissell: Arch. Surg. *36*: 53 (1938).
620. A. Brunschwig: Am. J. Cancer *34*: 540 (1938).
620a. H. L. Stewart & E. Lorenz: J. Nat. Cancer Inst. *2*: 193 (1941).

the development of a great number of sarcomas and gliomas which could be subcutaneously transplanted into other animals.[620b] It is noteworthy that cases have been recorded of tumor formation in laboratory workers handling these powerful carcinogenic substances; an epithelioma of the forearm was caused by benzpyrene,[621] a squamous-cell sarcoma of the nasolabial fold by methylcholanthrene.[622] It is obviously proven by the reports mentioned that the tumorigenic action of the substances under discussion is therefore not confined to any particular organ or tissue, but may have effect wherever they reach a sufficient concentration and meet with favorable conditions.

Several studies have dealt with the excretion and the metabolism of the carcinogenic hydrocarbons. In contrast to earlier negative findings,[623] later work succeeded in proving the presence of dibenzanthracene in tumors produced by this substance.[624] Dibenzanthracene, dissolved in fat and injected intraperitoneally into mice, was found to disappear rapidly, whereas colloidal watery solutions were resorbed somewhat more slowly; the hydrocarbon could not be detected in the excreta.[625] Dibenzanthracene, benzpyrene and methylcholanthrene, administered to mice, rabbits and fowl, were likewise noted as disappearing several hours or days following the injections, and they could not be detected in excreta or secretions, excepting the bile, but were found in the liver.[626, 627] A study on the excretion of benzpyrene in rats showed that approximately one per cent was excreted unchanged in the feces, while the rest was either converted to derivatives or remained at the site of the injection.[628] After application of carcinogenic hydrocarbons to mice, the excretion of photo-dynamic substances in the urine was observed and, moreover, a close correlation between the carcinogenic potency of the substance employed and the intensity of the photo-dynamic action of the urine was inferred. For this reason, investigation of the urine of cancer patients has been proposed.[629] This finding is the more interesting, as it has been reported that carcinogenic hydrocarbons strongly sensitize the skin of mice against light,[630] and exert on paramecia a photo-dynamic effect which is superior to that of noncarcinogenic substances (eosin, acridine, aniline sulfate).[631] Tissue cultures of chick-embryo cells, to which carcinogenic hydrocarbons had been added, were also arrested in growth if exposed to light.[632] Highly interesting results were obtained in studies in which feces of animals which had received injections of dibenzanthracene were analyzed.[633] From pooled feces of rabbits a phenolic derivative has been isolated, which proved to be noncarcinogenic and was subsequently identified as being 4′, 8′-dihydroxy–1, 2, 5, 6-dibenzanthracene.[634] Pooled excreta of mice, or rats, respectively, yielded dibenzanthracene derivatives of a different spectral behavior, but yielded as well unchanged dibenzanthracene. In continuation of this work, dibenzanthracene was administered to mice, rats, rabbits, dogs and monkeys, and the metabolites of the carcinogen were isolated from urine, feces, bile and tissues of the animals.

[620b] H. M. Zimmerman & H. Arnold: Cancer Research *1*: 919 (1941).

[621] E. Klar: Klin. Wchnschr. *17*: 1279 (1938).

[622] T. Gordonoff & B. Walthard: Schweiz. med. Wchnschr. *69*: 381 (1939).

[623] J. G. Chalmers: Biochem. J. *28*: 1214 (1934).

[624] E. Lorenz & M. J. Shear: Am. J. Cancer *26*: 333 (1936).

[625] I. Berenblum & L. P. Kendal: Biochem. J. *30*: 429 (1936).

[626] J. G. Chalmers & P. R. Peacock: Biochem. J. *30*: 1242 (1936).

[627] P. R. Peacock: Brit. J. Exper. Path. *17*: 164 (1936).

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628. J. G. Chalmers & A. H. M. Kirby: Biochem. J. *34*: 1191 (1940).
629. J. C. Mottram: Proc. Roy. Soc. London, s. B *126*: 560 (1939).
630. I. Domiach & J. C. Mottram: Nature, London *140*: 588 (1937).
631. I. Domiach: Brit. J. Exper. Path. *20*: *227* (1939).
632. M. R. Lewis: Am. J. Cancer *25*: 305 (1935).
633. K. Dobriner, C. P. Rhoads & G. I. Lavin: Proc. Soc. Exper. Biol. & Med. *41*: *67* (1939).
634. J. Cason & L. F. Fieser: J. Am. Chem. Soc. *62*: 2681 (1940).

Small quantities of unchanged dibenzanthracene were found to be excreted in the urines of mice, rats and rabbits and, in addition, phenolic derivatives of the hydrocarbon were detected in the same substrates. By means of spectroscopy, it was ascertained that the substances excreted by mice and rats were identical, but they differed from those excreted by rabbits. Since, furthermore, the dihydroxy-dibenzanthracene formed by rabbits proved to be noncarcinogenic for mice, it may be inferred that rabbits are endowed with the capacity of converting a carcinogen into a noncarcinogenic compound by means of hydroxylation. These circumstances may have direct bearing on the relative carcinogenicity of dibenzanthracene in different animal species.[634a] According to another study, rats injected with dibenzanthracene excreted both the unchanged hydrocarbon and 4′, 8′-dihydroxy–1, 2, 5, 6-dibenzanthracene.[634b]

Numerous comprehensive studies have been devoted to a continued analysis of the chemical structure of the polycyclic substances discussed in relation to their tumorigenic property. The phenomenon of a characteristic fluorescence spectrum, as mentioned above, proved to be an extremely valuable method for the recognition and isolation of carcinogenic substances of the polycyclic series; the usefulness of this method has been sustained,[635] in spite of objections raised which claimed that the spectra of benzpyrene and other hydrocarbons were markedly changed by the presence of other substances, so as to make it difficult to evaluate the results.[636] Nevertheless, a subsequent study demonstrated that carcinogenic substances could be isolated by means of spectrographic

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634a. K. Dobriner, C. P. Rhoads & G. I. Lavin: Cancer Research *2*: 95 (1942).

634b. R. N. Jones: Cancer Research *2*: 245 (1942).

635. W. V. Mayneord: Biochem. J. *30*: 707 (1936).

636. C. Sannié: Biochem. J. *30*: 704 (1936).

methods.[637] The fluorescence intensity, however, of C_{20}-homologs of cholanthrene was not found consistently to express the carcinogenic activity of these compounds.[638] A very striking phenomenon became apparent from the comparison of all three groups of carcinogenic hydrocarbons discussed so far, that is, that all of them, the derivatives of 1, 2-benzanthracene, 3, 4-benzpyrene and cholanthrene, contain a common structure in the phenanthrene nucleus, as may be seen from Figure I. Further interesting relations have been revealed by studies which examined the influence of substitutions performed at various positions of the ring. Thus it has been shown that the only slightly potent 1, 2-benzanthracene could be converted into highly active carcinogenic compounds by alkyl substitution in the 5 or 6 position; the latter substitution was less effective than the former, but 5-alkyl substitutions led to more potent compounds if the size of the substituent was increased. Substitutions in other positions were not only found ineffective, but even decreased the carcinogenic activity.[604] In this connection it will be recalled that the highly potent 1, 2, 5, 6-dibenzanthracene fits well into this scheme. Continued studies demonstrated that substitution also in the 9 and 10 position resulted in the synthesis of very active compounds.[600, 639] The effectiveness of the introduction of new groups into the benzanthracene molecule was found to decrease in the following order: 10, 5, 9, 6, and in fact a great carcinogenic potency of 9, 10-dimethyl-1, 2-benzanthracene was observed in mice.[640, 641] Lately, more

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637. F. Almassy: Biochem. Ztschr. *291*: 421 (1937).
638. N. F. Bruce: J. Am. Chem. Soc. *63*: 304 (1941).
639. W. E. Bachmann, J. W. Cook, A. Dansi, C. G. M. de Worms, G. A. D. Haslewood, C. L. Hewett & A. M. Robinson: Proc. Roy. Soc. London, s. B *123*: 343 (1937).
640. W. E. Bachmann, E. L. Kennaway & N. M. Kennaway: Yale J. Biol. & Med. *11*: 97 (1938).
641. M. J. Shear: Am. J. Cancer *33*: 499 (1938).

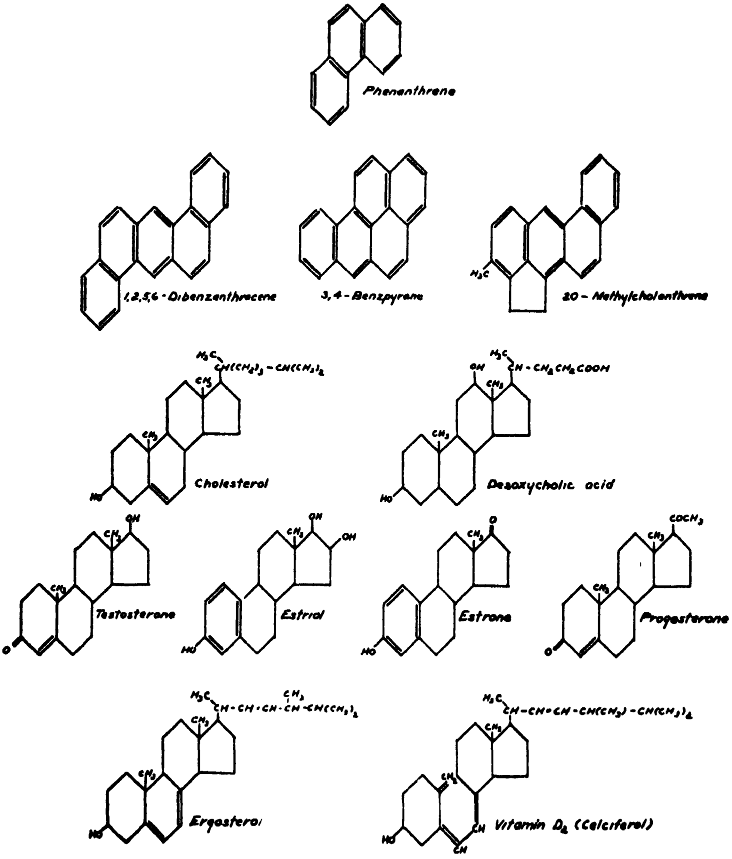
Phenanthrene
1,2,5,6-Dibenzanthracene
3,4-Benzpyrene
H_3C
20-Methylcholanthrene
H_3C
$CH(CH_2)_3-CH(CH_3)_2$
CH_3
CH_3
HO
Cholesterol
H_3C
OH
$CH-CH_2CH_2COOH$
CH_3
CH_3
HO
Desoxycholic acid
OH
CH_3
CH_3
O
Testosterone
OH
CH_3
OH
HO
Estriol
O
CH_3
HO
Estrone
$COCH_3$
CH_3
CH_3
O
Progesterone
H_3C
CH_3
$CH-CH=CH-CH-CH(CH_3)_2$
CH_3
CH_3
HO
Ergosterol
H_3C
$CH-CH=CH-CH(CH_3)-CH(CH_3)_2$
CH_3
CH_2
CH
HO
CH
Vitamin D_2 (Calciferol)

FIGURE 1

than seventy carcinogenic compounds have been investigated as to their structural relationship,[642] and twenty-four active benzanthracene derivatives were found among sixty-six synthesized and tested.[643] Forty-five additional compounds were tested by means of subcutaneous injections into mice and a carcinogenic quality was demonstrated for some new derivatives of 1, 2-benzanthracene and of 3, 4-benzphenanthrene.[643a] Likewise a number of new compounds related to methylcholanthrene were shown to be tumorigenic.[643b] The quantitative determination of the activity of methylcholanthrene, benzpyrene and dibenzanthracene resulted in grouping them in the order mentioned[644] and a special technique has been devised for the biologic standardization of carcinogens.[645] A very thorough analysis of his own experiments and of the results reported by other authors has been performed by Fieser,[646] who compared the relative potency of carcinogenic compounds with their structural relationship and analyzed what the influence is of variable experimental factors, such as the dose and physical state of the substances tested, and the age, sex, strain, species and tissue of the animals employed. The author associated the carcinogenic activity with specific types of chemical reactivity, as expressed by the ability of the hydrocarbons to undergo coupling with diazo compounds, and to react with a selective oxidation at specific molecular centers when treated with lead tetraacetate

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[642] G. M. Badger, J. W. Cook, C. L. Hewett, E. L. Kennaway, N. M. Kennaway, R. H. Martin & A. M. Robinson: Proc. Roy. Soc. London, s. B *129*: 439 (1940).

[643] M. J. Shear, J. Leiter & A. Perrault: J. Nat. Cancer Inst. *1*: 303 (1940).

[643a] M. J. Shear & J. Leiter: J. Nat. Cancer Inst. *2*: 241 (1941).

[643b] M. J. Shear: J. Nat. Cancer Inst. *2*: 99 (1941).

[644] M. B. Shimkin & H. B. Andervont: J. Nat. Cancer Inst. *1*: 45 (1940).

[645] M. B. Shimkin: J. Nat. Cancer Inst. *1*: 211 (1940).

[646] L. F. Fieser: Am. J. Cancer *34*: 37 (1938).

(formation of 10-acetoxy compounds). However, the general validity of this rule has been doubted since not all carcinogenic compounds seem to follow it.[607] In a recent study on several new derivatives of dibenzanthracene and of methylcholanthrene, the use of acetone as solvent for these compounds was found to enhance their carcinogenic activity.[646a]

The establishment of general rules governing the carcinogenic property of polycyclic hydocarbons has been further complicated by the discovery of a number of compounds, which deviate in their chemical structure from the substances named. 1, 2, 5, 6-dibenzfluorene was found slightly tumorigenic,[574] likewise chrysen and some of its derivatives, 3, 4,-5, 6-dibenzacridine,[604, 647] 2,methyl–3, 4-benzphenanthrene [639] and 3, 4, 5, 6-dibenzcarbazole.[648, 649] Injections of the latter substance into mice were disclosed as resulting in subcutaneous sarcomas or in pulmonary tumors; different mouse strains were distinguished as to their response to this carcinogen.[649a] While these substances are still structurally related to the derivatives of benzanthracene, a carcinogenic quality has been ascribed also to some compounds of an entirely different constitution, i.e., to s-triphenylbenzene and to tetraphenylmethane; it was reported that painting of mice with these substances resulted in malignant neoplasms.[650, 651] However, other experiments with these compounds led to negative results,[639] and no other confirmatory reports have

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646a. J. T. Bradbury, W. E. Bachmann & M. G. Lewisohn: Cancer Research *1*: 685 (1941).

647. P. Rondoni & A. Corbellini: Tumori n. s. *10*: 106 (1936).

648. E. Boyland & A. M. Brues: Proc. Roy. Soc. London, s. B *122*: 429 (1937).

649. L. C. Strong, G. M. Smith & W. U. Gardner: Yale J. Biol. & Med. *10*: 335 (1938).

649a. H. B. Andervont: J. Nat. Cancer Inst. *2*: 139 (1941).

650. A. A. Morton, D. B. Clapp & C. F. Branch: Science *82*: 134 (1935).

651. A. A. Morton, C. F. Branch & D. B. Clapp: Am. J. Cancer *26*: 754 (1936).

as yet been published. A discussion of several azo compounds which exhibit a carcinogenic activity will be given later on.

Under these circumstances it is therefore not surprising that endeavors to define the mechanism responsible for the tumor production by these substances met with considerable difficulties. Employing vital staining with phenol red, the precancerous skin lesions of mice, produced by carcinogenic hydrocarbons, have been investigated, and a staining behavior differing from that of normal skin was observed;[652] but it appears likely that this alteration was caused by vascular disturbances. In spite of the fact that various carcinogenic substances seemed to influence the respiration and glycolysis of organs, it has been questioned whether an interference with oxidation is primarily responsible for the induction of malignancy by the carcinogenic compounds, since it was found that benzpyrene decreased the oxidation only after tumor development had set in.[653] In this connection, it has also been ascertained that the capacity of hydrocarbons for undergoing reversible photo-oxidation is not related to their carcinogenicity,[654, 655] nor could a definite relationship be established between the tumorigenic action and the influence on oxidations, when measured by polarographic methods.[656] An inhibition of phospholipid oxidation in vitro has been observed following the addition of carcinogenic hydrocarbons, but since many other compounds also acted similarly, it is doubtful whether this effect is connected with the specific carcinogenic mechanism.[657] As far as local factors are concerned, it has been reported that the sarcoma formation, following benzpyrene injection, depended less on the local tissue reaction than on the absorption rate, i.e., the tumor incidence increased inversely to the speed of the disappearance of the hydrocarbon.[658] Acute inflammation produced by turpentine oil failed to influence the benzpyrene tumor development in mice,[659] but the basic fraction of creosote oil,[660] or croton oil and croton resin, were described as enhancing the tumor development following benzpyrene application to mice,[661] in contrast to an inhibiting effect of mustard gas.[662] These "cocarcinogenic" and "anti-carcinogenic" actions may be taken as being indications of an interference with certain metabolic processes, initiated by the hydrocarbons during the transformation of a normal into a malignant cell. In this respect, great importance has been attributed to sulfhydryl and cysteine derivatives of carcinogenic hydrocarbons, which were assumed to be formed in the body and to interact with cellular constituents during the process of cancerization.[663] The interaction of sterols and of carcinogenic hydrocarbons has been studied by means of surface films, and since even slight structural changes of the compounds investigated caused marked alterations of the type and intensity of attraction, it was believed that these relations might be of great importance in regard to the relationship between chemical structure and carcinogenic potency.[664, 665] On the basis of histologic analyses, it has been assumed that the carcinogenic hydrocarbons were selectively localized in the mitochondria of the cells, and were there exerting a decisive influence on the cell metabolism.[666] Experiments were reported recently which were undertaken for the purpose of clarifying the fundamental question as to whether the cancerization was necessarily preceded by necrotic changes of the tissues. In this study, injections of minced spleen (necrotic tissue) were administered to mice prior to the administration of methylcholanthrene, but since no significant influence on the tumor development was observed, a decision on this problem could not be reached.[667] An interesting theory was propounded on the basis of histologic examinations of the skin of mice after external application of methylcholanthrene. The findings suggested that the carcinogen at first inhibited the mitotic activity of the skin tissue, this having been injured by the toxic agent. In a later stage, however, this toxic effect was replaced by an active stimulation of cellular proliferation. This phenomenon was believed to be due to a substance ("proliferin") formed by the tissue in response to the carcinogen. Since this proliferation of the skin tissue was found to persist for a long time, it appeared reasonable to assume that frequent, short-interval application of carcinogens would not be the best method for inducing carcinogenesis. As a matter of fact, it was demonstrated that infrequent, long-interval administration of methylcholanthrene required much smaller doses of the carcinogen than the other method, although the latent

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[652] J. W. Orr: J. Path. & Bact. *46*: 495 (1938); *49*: 157 (1939).

[653] L. Th. Larionow: 3rd Internat. Cancer Congress, Atlantic City, p. 115 (1939).

[654] L. Velluz: Compt. rend. Acad. d. sc. *206*: 1514 (1938).

[655] J. W. Cook, R. Martin & E. M. F. Roe: Nature, London *143*: 1020 (1939).

[656] N. Waterman: Acta brev. Neerland. *9*: 143 (1939); Compt. rend. Soc. de biol. *130*: 1115 (1939).

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[657] H. P. Rusch & B. E. Kline: Cancer Res. *1*: 465 (1941).
[658] P. R. Peacock & S. Beck: Brit. J. Exper. Path. *19*: 315 (1938).
[659] S. Beck: Brit. J. Exper. Path. *19*: 319 (1938).
[660] R. D. Sall: J. Nat. Cancer Inst. *1*: 45 (1940).
[661] I. Berenblum: Cancer Res. *1*: 44; 807 (1941).
[662] I. Berenblum: J. Path. & Bact. *32*: 425 (1929).
[663] J. L. Wood & L. F. Fieser: J. Am. Chem. Soc. *62*: 2674 (1940).

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[664] G. H. A. Clowes, W. W. Davis & M. E. Krahl: Am. J. Cancer *36*: 98 (1939); *37*: 453 (1939).

[665] W. W. Davis, M. E. Krahl & G. H. A. Clowes: J. Am. Chem. Soc. *62*: 3080 (1940).

[666] A. Graffi: Ztschr. f. Krebsforsch. *49*: 477 (1939); *50*: 196 (1940).

[667] W. H. Woglom: Am. J. Cancer *40*: 429 (1940).

period was prolonged. No indication can be offered as yet as to the nature of the "proliferin" formed by tissues exposed to carcinogens, but the authors of this study felt justified in assuming that the resistance or susceptibility of animals to tumor development may depend, in some instances, upon the tendency to form this proliferin.[667a]

It is in complete accordance with the generally prevailing conception, which envisages neoplasia as an affection of the whole body and not merely as a local disease, that many observations proved systemic effects to be exerted by the carcinogenic substances, apart from the local tumor formation. It is, however, by no means established to what degree these generalized alterations are directly connected with the carcinogenic action, or whether they represent incidental effects only. The hematopoietic apparatus appears greatly to be affected, as indicated by quantitative and qualitative changes of the white blood count observed in animals subjected to treatment with carcinogenic hydrocarbons.[668] A myeloid metaplasia of the spleen was observed in mice after painting their skin with dibenzanthracene,[577] an atrophy of the spleen resulted from benzpyrene injections into rats [583] and, similarly, atrophy of the spleen, bone marrow, lymph nodes and thymus was observed in mice which had received subcutaneous or intraperitoneal benzpyrene injections.[669, 670] In mice treated with benzpyrene, an amyloidosis of spleen and liver has been noted prior to the development of tumors, and this damage resulting in the reticulo-endothelial system

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667a. W. Cramer & R. E. Stowell: Cancer Research *1*: 849 (1941); J. Nat. Cancer Inst. *2*: 369; 379 (1942).

668. L. D. Parsons: J. Path. & Bact. *40*: 45 (1935); *43*: 1 (1936); *47*: 501 (1938).

669. E. Picard & H. Laduron: Compt. rend. Soc. de biol. *115*: 1739 (1934).

670. E. Picard: 2nd Internat. Cancer Congress, Brussels *2*: 120 (1936).

was considered as favoring the initiation of neoplasia.[671] Oral ingestion of dibenzanthracene or methylcholanthrene was also followed by an atrophy of the spleen and of the hematopoietic organs,[617] while other workers found a hyaline degeneration of the spleen in animals treated with carcinogenic hydrocarbons and, at the same time, an enlargement of this organ, approximately proportional to the carcinogenic potency of the compound used: The spleen of benzpyrene-treated animals was approximately five times larger than the organ of animals treated with dibenzanthracene, and methylcholathrene caused twice the enlargement of that of benzpyrene.[672] Direct evidence for an impairment of the reticulo-endothelial system, due to the action of carcinogenic hydrocarbons, seems to be furnished by experiments which determined the function of this system in rabbits by means of the Congo Red test before, during, and after prolonged administration of these compounds, and which showed a definite decrease of the reticulo-endothelial activity as resulting from the treatment.[673] Significantly, noncarcinogenic hydrocarbons of closely related chemical structure did not, or did much less markedly, exert such an effect.[674]

An entirely different mechanism is obviously involved in the growth inhibition observed in young rats which were fed methylcholanthrene or benzpyrene. This impairment of normal growth could be suppressed by the addition of sulfhydryl compounds to the diet, which seems to indicate that these substances, necessary for normal growth, are excessively consumed under the influence of the ingested hy-

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671. X. Chahovitch, M. Vichnjitch & J. Ignjatchev: Libro de Oro, Buenos Aires, p. 206 (1936).

672. J. M. Twort & C. C. Twort: Am. J. Cancer *28*: 78 (1937).

673. T. Csato, C. Wetzler-Ligeti & B. P. Wiesner: Am. J. Cancer *36*: 257 (1939).

674. C. Hoch-Ligeti: Am. J. Cancer *39*: 365 (1940).

drocarbons, probably for the purpose of detoxicating them.[675] Furthermore, since no essential difference was noted between carcinogenic and noncarcinogenic compounds, as to their influence on normal growth,[676] it may be assumed that this action is solely the consequence of their toxicity. A similar interpretation is likely to be adequate for the finding that uterine and placentar hemorrhage, growth cessation and resorption of fetus and placenta occurred in pregnant rats when they received injections of dibenzanthracene, benzpyrene, and also of the weakly carcinogenic 1, 2-benzanthracene.[677]

Although it has not yet been possible to define exactly and to evaluate the systemic effects accompanying, or even determining the cancerization initiated by the substances under discussion, it may be postulated that such a participation of the organism is a necessary requirement for the tumor development. This view seems to be supported by the fact that only one author claimed he had achieved the transformation of normal cells into malignant ones in tissue cultures—i.e., outside of the body—by means of carcinogenic hydrocarbons,[678] while all other studies so far failed to accomplish such a result. Methylcholanthrene, or dibenzanthracene added to cultures of rat and mouse fibroblasts, caused a growth cessation and degenerative changes of the cells,[679] whereas in other experiments carcinogenic hydrocarbons stimulated the growth, if applied in very weak concentrations, and suppressed it, if larger amounts were added.[680]

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[675] J. White & A. White: J. Biol. Chem. *131*: 149 (1939); Proc. Soc. Exper. Biol. & Med. *39*: 527 (1938).

[676] A. White & J. White: Yale J. Biol. & Med. *12*: 427 (1940).

[677] J. M. Wolfe & W. R. Bryan: Am. J. Cancer *36*: 359 (1939).

[678] M. des Ligneris: Compt. rend. Soc. de biol. *120*: 777 (1935); *121*: 1579 (1936).

[679] W. R. Earle & C. Voegtlin: Am. J. Cancer *34*: 373 (1938).

[680] E. M. H. Creech: Am. J. Cancer *35*: 191 (1939); *39*: 149 (1940).

It may impress one as paradoxical that in the following survey the powerful carcinogenic hydrocarbons will be described as exerting just the opposite action, i.e., as inhibiting or counteracting malignant growth; but at the same time it will be remembered that a similar ambivalent behavior has also been encountered in the sphere of inorganic chemistry where the tumor producing, and also the tumor curing, actions of arsenic and of radio-active substances have been discussed. The first report of this kind, referring to hydrocarbons, was based on experiments with the Jensen rat sarcoma, the growth of which was found to be inhibited by intraperitoneal injections of colloidal solutions of dibenzanthracene, benzpyrene, and also, to a lesser degree, of weakly active or entirely noncarcinogenic related substances.[681] Likewise, the growth of spontaneous mouse carcinomas was seen to be retarded by dibenzanthracene, but tumor regressions observed in some of the treated animals were followed by recurrences after four weeks.[682] A very marked effect from intraperitoneal dibenzanthracene-lecithin injections has been found in a transplantable rat sarcoma of high resistance: Twenty tumors out of fifty-one treated animals disappeared completely and many others were slowed down in their growth, whereas spontaneous tumor regressions never occurred in control animals.[683] An inhibition or even complete regression of spontaneous mouse tumors has been achieved by parenteral administration of various hydrocarbons, and it is noteworthy that the effect of noncarcinogenic compounds was slight, or very temporary;[684] a slight growth inhibition was exerted by hydrocarbons also

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681. A. Haddow: Nature, London *136*: 868 (1935); Proc. Roy. Soc. London, s. B *122*: 442 (1937).

682. F. C. Pybus & E. W. Miller: Brit. J. Exper. Pathol. *18*: 126 (1937).

683. F. A. McJunkin & W. Wolavka: Arch. Path. *25*: 506 (1938).

684. A. Haddow: J. Path. & Bact. *47*: 567 (1938).

on those tumors which had been originally produced by the same substances.[685] Thirty-four compounds of known carcinogenic power have been subsequently tested on spontaneous breast cancers of mice, on Crocker mouse sarcoma 180, and on Walker rat carcinoma 256, with the result that 86.5 per cent of the experiments showed a growth inhibition, whereas experiments performed with thirty-four supposedly noncarcinogenic compounds failed to inhibit the tumor growth in 79.7 per cent of the animals.[686] Intravenous benzpyrene injections inhibited the growth of a transplanted mouse carcinoma, if the dosage employed was sufficiently large: six out of thirteen tumors regressed. The toxicity of benzpyrene was found to be highest for rats and goats, less for fowl, mice and humans.[687] 1, 2, 5, 6-dibenzfluorene also was seen strongly to inhibit the growth of spontaneous mouse carcinoma, and it was stressed that this property, and the carcinogenicity of a compound, do not necessarily run parallel.[688] A tumor growth inhibiting effect of benzpyrene[689] and of dibenzanthracene[690] was also observed in other studies, although no real cures or life prolongations were obtained.[690] In complete contrast to the reports mentioned, one author failed to notice a growth inhibition of Jensen rat sarcoma following intraperitoneal or subcutaneous benzpyrene injections.[691] Intraperitoneal injections of 9, 10-dimethyl-1, 2-benzanthracene dissolved in oil were given to mice bearing transplanted mammary carcinomas or Crocker sarcomas 180. A distinct growth inhibition of the latter tumor

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685. A. Haddow: J. Path. & Bact. *47*: 581 (1938).
686. A. Haddow & A. M. Robinson: Proc. Roy. Soc. London, s. B *127*: 277 (1939).
687. P. R. Peacock & S. Beck: Brit. J. Exper. Path. *19*: 434 (1938).
688. A. Haddow: 3rd Internat. Cancer Congress, Atlantic City, p. 117 (1939).
689. Ch. J. Keller & O. Künzel: Ztschr. f. klin. Med. *134*: 286 (1938).
690. J. C. Lees: Quarterly J. Exper. Physiol. *27*: 161; 171; 181 (1937).
691. B. Rarei & H. Gummel: Ztschr. f. Krebsforsch. *48*: 355 (1939).

resulted from this treatment, whereas the former tumor was not affected. It was stressed that the loss of body weight accompanying the injections of the hydrocarbon must be taken into consideration; therefore, the growth rate of tumors in treated animals was compared with control tumors, the hosts of which were kept on a calorie-restricted diet and exhibited approximately the same weight as the treated animals.[692] Prophylactic treatment of mice with benzpyrene, methylcholanthrene or dibenzanthracene, resulted in only a very weak protection against subsequent tumor inoculation, and was completely ineffective in rats.[693] In rabbits which had been treated for six weeks with twice weekly injections of dibenzanthracene, a subsequent transmission of Brown-Pearce carcinoma resulted in an increased yield of tumors, accelerated growth and enhanced formation of metastases.[694] For these divergences several factors may be responsible, such as differences of animal species and strains, tumor material and compounds used. The solvents employed may also be of great importance, as demonstrated by a study in which a considerable growth inhibition of the Walker rat carcinoma was observed, if the animals received injections of benzpyrene or dibenzanthracene dissolved in hog lard, whereas colloidal solutions were less effective, and agents dissolved in rat fat entirely ineffective.[695] It appears suggestive to correlate the growth inhibition of tumors with the analogous effect on normal growth exerted by the hydrocarbons. However, it has not yet been clarified whether these two actions are parallel, and whether carcinogenicity and growth inhibition are fundamentally connected appears subject to a divergence of opinion.[675, 676, 686] It is, however, not acceptable to ascribe the tumor inhibiting action of the hydrocarbons solely to a toxic, necrotizing effect, as it has been deemed on the basis of experiments using intratumoral injections of the hydrocarbons [696, 697] since this procedure has not been employed in the investigations enumerated. On the other hand, the lack of a satisfactory experimental elaboration of this problem does not seem as yet to warrant a clinical use of carcinogenic hydrocarbons for therapeutic purposes, as has been attempted in a few cases with more or less impressive results.[689, 698, 699] A different approach which might lead to some therapeutic results has been chosen by authors who prepared a dibenzanthracene-protein complex, which still retained part of the carcinogenic potency; the authors were able to evoke with this compound a definite immunologic reaction as demonstrated by precipitin tests.[700] However only from continued work can more valid conclusions be derived in this respect.

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[692] S. Stamer: Acta path. et microbiol. Scandinav. *18*: 533 (1941).

[693] H. Alapy: Ztschr. f. Krebsforsch. *48*: 32 (1938).

[694] M. Appel, A. A. Strauss, G. Kolischer & H. Necheles: Am. J. Cancer *33*: 239 (1938).

[695] E. Morelli & A. Dansi: Nature, London *143*: 1021 (1939).

If an attempt is now made to summarize and to evaluate the principal results of the work discussed, it becomes obvious that two positive accomplishments must be acknowledged. One, the unquestionably meritorious elucidation of the chemical nature of certain organic carcinogens by the isolating of the responsible compounds in pure form, identification of their structure, and the enabling of the preparation of new synthetic products endowed with high carcinogenic potency; and, two, the enrichment of experimental cancer research by making available these powerful and well defined tumorigenic agents. But one problem of pre-eminent significance is still awaiting final solution; i.e., whether the

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696. N. Brock, H. Druckrey & H. Hamperl: Arch. f. exper. Path. u. Pharmakol. *189*: 709 (1938); Arch. f. klin. Chir. *194*: 250 (1939).

697. H. J. Lauber, E. Hildebrand & H. Schocke: Ztschr. f. d. ges. exper. Med. *105*: 370 (1939).

698. K. H. Bauer: Arch. f. klin. Chir. *189*: 123 (1937).

699. G. B. Cottini & G. B. Mazzoni: Am. J. Cancer *37*: 186 (1939).

700. H. J. Creech & W. R. Franks: Am. J. Cancer *30*: 555 (1937).

carcinogenic compounds discussed represent merely one of the several known exogenous causative factors of neoplasia, standing, so to speak, at the periphery of the etiology, or whether they may be assigned a more central position, by assuming also their essential involvement in tumor formation of other, known or unknown, etiology. The latter view would necessarily presuppose an endogenous formation of substances, chemically related to the carcinogenic hydrocarbons, from normal constituents of the body under certain pathologic conditions. No conclusive evidence for the actual existence of such a phenomenon has been furnished so far, but its possibility seems at least hinted at, in consideration of the remarkable chemical resemblance to the carcinogens exhibited by some physiologic body constituents, such as sterols, bile acids and sex hormones. All the substances named have the phenanthrene nucleus in common, which, as described, is also contained in most of the carcinogenic hydrocarbons. It must, however, be recalled that of the innumerable phenanthrene derivatives, a comparatively small number only, exhibiting a specific chemical constitution, is endowed with tumorigenic property, and in this respect it is notable that, for instance, cardiac glucosides and saponins of phenanthrene structure were found to be devoid of a tumor producing activity.[701, 702] On the other hand, experimental work has succeeded, in the case of methylcholanthrene, in synthesizing this potent carcinogen from physiologic body constituents, such as bile acids [570, 571] and, more recently also, from the sterol compounds cholestanone and cholestenone,[703] thus leaving open the possibility of similar conversions taking place in the body. Other investigations which attempted to

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[701] P. K. Smith & W. U. Gardner: Yale J. Biol. & Med. *11*: 187 (1939).

[702] H. Druckrey: Ztschr. f. Krebsforsch. *50*: 27 (1940).

[703] W. Rossner: Ztschr. f. physiol. Chem. *249*: 267 (1937).

derive carcinogenic products from cholesterol by various methods, as discussed above, seem to be of less convincing character and are in need of further elaboration. Another correlation between the carcinogenic hydrocarbons and physiologic substances concerned the female sex hormones, and has been much discussed because, on the one hand, it was observed that some synthetic carcinogenic compounds exerted an estrogenic effect in mice [704] and, on the other hand, because it received support from extensive work demonstrating a tumor promoting effect and, under special conditions, even a carcinogenic action of the estrogens themselves. Although the discussion of the latter phenomenon is reserved for the chapter on hormones, it may suffice here to point out that, according to authoritative views, no direct interdependence exists between carcinogenic and estrogenic action,[705] and that, furthermore, the tumor-favoring action of female sex hormones is essentially different from the carcinogenesis initiated by the hydrocarbons named.[705-707] A theory of broad biological implications has been conceived on the basis of experiments in which the carcinogenic hydrocarbons were found to influence the embryonic development of lower animals, and they were therefore grouped in the class of "evokators" and "organizers." [708, 709] It was assumed that the carcinogenic substances and related endogenous products might bring about a disturbance of the normal growth pattern and thus initiate malignancy. But since a great variety of chemically different substances have been shown to affect the embryonic development in a similar manner,[710] one would seem not justified in drawing such general conclusions from these experimental observations.

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[704] E. C. Dodds: 1st Internat. Cancer Congress, Madrid, *2*: 181 (1933).

[705] L. Loeb: Glandular Physiology and Therapy, A. M. A., Chicago 1935, p. 177.

[706] A. Lacassagne: 3rd Internat. Cancer Congress, Atlantic City (1939).

[707] J. J. Morton: Surg. Gynec. & Obst. *72*: 345 (1941).

[708] C. H. Waddington: Nature, London *135*: 606 (1935).

[709] J. Needham: Brit. M. J. *2*: 701 (1936); Proc. Roy. Soc. Med. *29*: 1577 (1936).

Aniline cancer and carcinogenic azo compounds. Here again it was an occupational disease which called the attention of clinicians, and later on also of research workers, to a tumorigenic activity of certain nitrogenous aromatic substances. The rapid development of the synthetic dye industry, mainly employing aniline derivatives as basic materials, which was inaugurated at the end of the last century, was soon followed by observations of a disquietingly high frequency of bladder cancer in the workmen occupied in these plants. Pertinent clinical experiences were recorded in 1912[711] and a great number of more recent publications dealt with the statistical analysis and clinical aspects of the tumors in workers in aniline plants.[712-715] The prevailing localization in the urinary tract, and here mainly in the bladder, was believed to result from the urinary excretion of the compounds or their conversion products, after they had entered the body by way of the skin and the lungs. The disease usually starts after a latent period of more than ten years with the formation of papillomas which undergo malignant degeneration, and which are very resistant to surgical therapy because of their great tendency to recurrences.[716] The substances which were deemed responsible for the tumor formation are, apart from aniline, benzidine and, especially, naphthylamines.[717] Efforts to imitate this occupational carcinogenesis in corresponding animal experiments at first yielded completely negative results.[718-722] But later it was reported that malignant bladder tumors arose in two rabbits which had been exposed to β-naphthylamine vapors for 20-24 months,[723] and malignant neoplasms of the urinary bladder were observed in seven out of seventy rabbits following subcutaneous injections of aniline or β-naphthylamine, respectively.[724] However, other authors expressed a critical attitude towards these claims, considering the histologic evidence furnished as unconvincing, and they themselves failed to achieve tumor formation in rabbits and rats by injecting or feeding various aniline derivatives.[725] The choice of suitable experimental animal species seems to be of great importance, since injections and feeding of β-naphthylamine were found to cause benign and also malignant bladder tumors in dogs,[726] but the same compound failed to evoke tumors in rabbits.[727] Similarly, the painting of the skin with hydrazobenzene, a product related to aniline, elicited tumor development in rats, but not in mice or rabbits.[728] Other studies employed less clear experimental arrangements, by combining the injection of embryonic cells with exposure to inhalation of β-naphthylamine, which treatment was claimed to result in the formation of cancer-like growth in the lung.[729] The evaluation of this result appears doubtful, and, likewise, another worker did not specify whether bladder papillomas produced in rabbits by injections of o-toluidine [730] eventually acquired malignant character. Other experiments tested several aniline dyes as to their influence on the tar cancer formation, and a promoting effect has been seen from chrysoidine (diaminoazobenzene).[731] A theoretical conception has been offered regarding the mechanism of tumor formation by aniline and related substances, by attributing to these chemicals a deleterious effect on growth-regulating enzymes,[732] but it is questionable whether any clear insight into the carcinogenic action may be gained from this rather vague explanation.

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[710] L. G. Barth: Physiol. Zoology *12*: 22 (1939).

[711] S. G. Leuenberger: Beitr. z. klin. Chir. *80*: 208 (1912); Wien. klin. Wchnschr. *26*: 433 (1913).

[712] L. W. Anderson: J. Urol. *31*: 148 (1934).

[713] D. M. Gay: J. Urol. *31*: 137 (1934); *38*: 221 (1937).

[714] R. S. Ferguson: J. Urol. *31*: 122 (1934).

[715] G. H. Gehrmann: J. Urol. *31*: 126 (1934); J. A. M. A. *107*: 1436 (1936).

[716] A. Müller: Ztschr. f. Urol. *25*: 411 (1931).

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[717] R. Oppenheimer: Ztschr. f. urol. Chir. *21*: 336 (1927).

[718] R. Oppenheimer: München. med. Wchnschr. *67*: 12 (1920).

[719] P. Schwerin: Zentralbl. f. Gewerbehyg. *8*: 64 (1920).

[720] H. Curschmann: Zentralbl. f. Gewerbehyg. *8*: 145; 169 (1920).

[721] R. Jaffé: Zentralbl. f. allg. Path. u. path. Anat. *31*: 57 (1920).

[722] C. Posner: Ztschr. f. Urol. *18*: 418 (1924).

[723] W. Schär: Cancer, Bruxelles *7*: 205 (1930); Deutsche Ztschr. f. Chir. *226*: 81 (1930).

[724] S. Perlmann & W. Staehler: Ztschr. f. Urol. *27*: 195 (1933).

[725] I. Berenblum & G. M. Bonser: J. Indust. Hyg. & Toxicol. *19*: 86 (1937).

[726] W. C. Hueper, F. H. Wiley & H. D. Wolfe: J. Indust. Hyg. & Toxicol. *20*: 46 (1938).

[727] W. C. Hueper, F. A. Briggs & H. D. Wolfe: J. Indust. Hyg. & Toxicol. *20*: 85 (1938).

[728] H. Auler: in C. Adams & H. Auler: Neuere Ergebnisse auf dem Gebiete der Krebskrankheiten, S. Hirzel, Leipzig, 1937, p. 100.

Compounds which have been obtained as by-products of dye fabrication in aniline plants are the dibenzcarbazoles, and it will be recalled that these nitrogen-containing hydrocarbons, structurally related to benzanthracene derivatives, have been mentioned in the preceding section, and have been recognized as tumorigenic for the skin of mice, 3, 4, 5, 6-dibenzcarbazole being the most active compound and also capable of producing hepatomas.[648, 649, 649a] Another condensed ring system, containing nitrogen, is β-anthraquinoline which, injected subcutaneously into rats, produced kidney carcinomas.[733]

A much greater amount of work has been devoted to investigations of azo compounds, products of dye character,

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729. R. Nicole: Ztschr. f. Krebsforsch. *43*: 173 (1936).
730. S. Morigami & I. Nisimura: Gann *34*: 146 (1937).
731. J. Forssman: Acta pathol. & microbiol. Scandinav. *8*: 16 (1931).
732. B. Bloch: Schweiz. med. Wchnschr. *57*: 1218 (1927).
733. A. Sempronj & E. Morelli: Am. J. Cancer *35*: 534 (1939).

industrially synthesized from aniline and its homologs. The oldest studies in this field concerned scarlet-red, a substance which causes a marked promotion of epithelial growth, and which has therefore been used extensively in ointments applied for the purpose of promoting epithelial proliferation. As long ago as 1906, it was reported that subcutaneous injections of oily solutions of scarlet-red, and also of sudan III, in rabbits resulted in atypical epithelial granulations.[734] These findings have been confirmed,[735, 736] but since only precancerous lesions were observed, and no true malignant neoplasms, these studies lapsed into the background, which is understandable considering the successful experimental work with other carcinogenic substances. Besides, peroral or subcutaneous administration of scarlet-red to mice did not even provoke the mentioned precancerous alterations.[737] But a new impetus for work with azo compounds has been received in more recent times from the results of authors [738-742] who administered the compound o-aminoazotoluene to rats. By feeding of a five per cent oily solution, or by injections of a ten per cent oily solution, malignant, transmissible hepatomas have been produced and skin tumors were also observed. Epitheliomas of the urinary bladder developed in rabbits fed o-aminoazotoluene,[743] and it was proved that this

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734. B. Fischer: München. med. Wchnschr. *53*: 1041 (1906); Zentralbl. f. allg. Path. u. path. Anat. *34*: 618 (1906).

735. L. Wacker & A. Schmincke: München. med. Wchnschr. *58*: 1607 (1911).

736. F. Klinge: Ergebn. d. inn. Med. u. Kinderh. *29*: 152 (1926).

737. C. T. Eckert, Z. K. Cooper & M. G. Seelig: Arch. Path. *19*: 83 (1935).

738. T. Yoshida: Tr. Jap. Path. Soc. *23*: 636 (1933); Gann *28*: 441; 445 (1934); Deutsche med. Wchnschr. No. 18 (1937).

739. Y. Nishiyama: Gann *29*: 1; 285 (1935).

740. T. Iikubo: Gann *29*: 79 (1935).

741. K. Miura: Bull. Assoc. franç. p. l'étude du cancer *24*: 534 (1935).

742. T. Sasaki & T. Yoshida: Virchows Arch. f. path. Anat. *295*: 175 (1935).

743. T. Yoshida: Gann *29*: 295 (1935).

compound was converted by the animals to, and excreted in the urine as, p-diacetyl toluylenediamine,[744] which substance might have been responsible for the bladder tumor formation. Likewise, feeding of o′-m′-dimethylazobenzene elicited development of bladder tumors.[745] Cholesterol and lecithin injections were found to enhance the hepatoma formation in rats fed o-aminoazotoluene [746] and, as mentioned before, subcutaneous glucose injections administered to rats fed with this compound brought about a sarcomatous growth at the site of the injection; but continued studies also showed the possibility of producing these tumors without the administration of aminoazotoluene.[72] Attempts, in Europe, to reproduce the results of the Japanese authors—production of hepatomas by means of o-aminoazotoluene—at first met with failure,[747] but later positive results were obtained,[748] provided the animals received a diet resembling that used by the original authors. In this country, several authors have published the results of successful experiments of hepatoma production by this substance in rats [749] or mice,[750, 751] and summarizing surveys on this problem were given in 1937.[603, 752] Among other azo compounds, dimethylaminoazobenzene (butter yellow), a dye stuff formerly used commercially for coloring of oils and butter, has been found to represent an effective carcinogen by producing hepatomas, which frequently metastasize, in rats fed this compound.[753]

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744. T. Hashimoto: Gann *29*: 306 (1935).
745. I. Otsuka & N. Nagao: Gann *30*: 561 (1936).
746. I. Hayashi & T. Tomita: Gann *31*: 232 (1937).
747. B. Fischer-Wasels: Verh. deutsch. path. Ges. *28*: 66 (1935).
748. B. Fischer-Wasels: Zentralbl. f. Path., Erg.-H. *66*: 182 (1937).
749. L. L. Water: Yale J. Biol. & Med. *10*: 179 (1937).
750. M. J. Shear: Proc. Am. Soc. Biol. Chem. 13th Meet., 100 (1936).
751. L. W. Law: Cancer Research *1*: 397 (1941).
752. M. J. Shear: Am. J. Cancer *29*: 269 (1937).
753. R. Kinosita: Osaka-Igaku Z. *35*: 403 (1936); Gann *30*: 423 (1936); Yale J. Biol. & Med. *12*: 287 (1940).

Analogous results were also obtained in mice, although this species seemed to be more resistant and required a longer period of feeding than rats.[754] These results have been confirmed [751, 754, 754a] and in an extensive study [755] it was demonstrated that dietary factors decisively influenced the development of the hepatomas due to butter yellow; a discussion of these aspects will be given in the chapter on nutrition and vitamins. A study of the metabolism of butter yellow in rats showed that the animals excreted the free and the acetylated forms of p-aminophenol and of p-phenylenediamine in the urine.[755a] The fact that metabolites of butter yellow are acetylated by the animals is significant in view of the findings that, after acetylation, these compounds do not any more inhibit the coenzyme I system in vitro.[755b] Hence acetylation of butter yellow metabolites may be considered a mechanism of detoxication protecting the body against interference with an important metabolic process.

It is reminiscent of the relations discussed in the preceding section that injections of o-aminoazotoluene markedly inhibited the growth of simultaneously inoculated Flexner-Jobling carcinoma in rats,[756] and that the growth of young rats was retarded by feeding of butter yellow, but resumed a normal rate when greater amounts of sulfhydryl compounds (cystine, methionine) were added to the diet.[757] Of related substances, some azonaphthalene compounds were found to produce hepatomas in mice [758] as do 2, 3′-azotoluene, 4′-hy-

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754. T. Mizuta & H. Maruya: Osaka-Igaku Z. *35*: 1187 (1936).

754a. J. E. Edwards & J. White: J. Nat. Cancer Inst. *2*: 157 (1941).

755. K. Sugiura & C. P. Rhoads: Cancer Research *1*: 3 (1941).

755a. E. S. Stevenson, K. Dobriner & C. P. Rhoads: Cancer Research *2*: 160 (1942).

755b. C. J. Kensler, S. O. Dexter & C. P. Rhoads: Cancer Research *2*: 1 (1942).

756. I. Hayashi & O. Kawase: Gann *30*: 461 (1936).

757. J. White: J. Nat. Cancer Inst. *1*: 337 (1940).

758. J. W. Cook, C. L. Hewett, E. L. Kennaway & N. M. Kennaway: Am. J. Cancer *40*: 62 (1940).

droxy–2, 3′-azotoluene.[751] The significance of the results obtained in the enumerated studies appears to be vested in the fact that internal tumors of genuine malignancy (hepatomas and bladder tumors) can be experimentally produced, and that peroral application is much more effective in these cases than in the case of the carcinogenic hydrocarbons. Furthermore, information is derived from them as to a possible blastogenic effect exhibited by products of widespread industrial and commercial use and, accordingly, protective steps against such health hazards may be undertaken.

In conjunction with the azo compounds, reference is made to a few other aromatic compounds containing nitrogen, to which a tumorigenic action has been ascribed. On the occasion of investigations dealing with trypanocide drugs, it has been observed that a complex quinoline derivative, "styryl 430," produced sarcomas when injected into mice, in ten out of nineteen animals after eight months.[759] [761] The vital stain "light green F. S.," a dye related to diethyldibenzyl diaminotriphenylcarbinol-trisulfonic acid, was injected into rats for about a year, after which time sarcomas developed at the site of the injections in four out of seven animals, but injections into mice failed to elicit tumors.[762] A carcinogen of higher potency has been found recently in 2-acetaminofluorene, a compound used as an insecticide. When this substance was incorporated into the diet of rats in a concentration of 0.031 per cent, epithelial hyperplasias appeared in the urinary bladder, the renal pelvis, the liver, the pancreas or the lungs of the animals after more than three months, and in 19 out of 39 animals, malignant tumors arose, exhibiting invasive

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759. C. H. Browning, J. B. Cohen, K. E. Cooper, S. Ellingworth & R. Gulbransen: Proc. Roy. Soc. London, s. B *113*: 300 (1933).

760. C. H. Browning, R. Gulbransen & J. S. F. Niven: J. Path. & Bact. *42*: 155 (1936).

761. C. H. Browning: 3rd Internat. Cancer Congress, Atlantic City, p. 135 (1939).

762. W. Schiller: Am. J. Cancer *31*: 486 (1937).

growth and, histologically, mostly carcinomatous character.[762a]

Substances which have acquired an eminent pharmacological significance in recent times are sulfanilamide and the related drugs. It is therefore of great interest that no carcinogenic effect has been observed in mice from sulfanilamide administration[763, 764] or from prontosil,[764] and it was also stated that sulfanilamide did not affect the growth of spontaneous or dibenzanthracene tumors of mice.[763] In another study, however, injection of sulfanilamide into mice was followed by invasive spindle cell growth in two out of twenty animals, but no metastases were observed, and feeding of the substance was found ineffective.[765] No clinical observations have so far been recorded which would suggest that these substances, widely used for several years, had induced malignant neoplasia.

Tobacco cancer. The experimental work on the carcinogenicity of tobacco and tobacco smoke is based on the well known occurrence of lip cancer in some smokers, particularly pipe smokers. Clinical and statistical observations on this disease have frequently been presented in recent years.[766-769] The animal experimentation, testing the carcinogenic action of tobacco, has been undertaken in two different ways. On the one hand, animals have been exposed to tobacco smoke for a prolonged time, and such a measure was claimed to

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762a. R. H. Wilson, F. deEds & A. J. Cox, Jr.: Cancer Research *1*: 595 (1941).

763. M. R. Lewis: Am. J. Cancer *34*: 431 (1938).

764. P. C. Zamecnik & S. Koletsky: Proc. Soc. Exper. Biol. & Med. *42*: 391 (1939).

765. A. T. Haerem: Proc. Soc. Exper. Biol. & Med. *45*: 536 (1940).

766. F. Lickint: Ztschr. f. Krebsforsch. *30*: 349 (1930).

767. G. Haase: Deutsche Monatschr. f. Zahnh. *49*: 881 (1931).

768. O. Schürch & A. Winterstein: Ztschr. f. Krebsforsch. *42*: 76 (1935).

769. F. L. Hoffman: Ann. Surg. *93*: 50 (1931).

have resulted in the development of lung tumors in a small percentage of the animals.[770] This finding, however, cannot be considered as conclusive evidence for a carcinogenic property of tobacco smoke, because no data have been given as to the incidence of spontaneous lung tumors in the respective strain, and a more exactly controlled study could not demonstrate an increased incidence of lung tumors in mice inhaling tobacco smoke.[550] Nevertheless, on the basis of a statistical analysis, one author [771] deemed the use of tobacco as being responsible for an increased frequency of lung tumors in human pathology. More evidence for a tumorigenic quality of tobacco products has been derived from experiments employing tobacco tar, a procedure which appears logical because, during smoking, tarry and resinous materials are formed and may come in contact with the lips and the respiratory tract of the smoker. Experiments in which tobacco tar has been applied to the skin of mice,[772] or of rabbits,[773] resulted in neoplastic changes only in a small percentage, or gave entirely negative results.[774] Only from one side [775] has it been claimed that tobacco tar preparations exhibited a strong carcinogenic potency. But these results might be due to the use of highly susceptible animal strains, and prevailing opinion considers tobacco tar only a weak carcinogen, as evidenced by the result of animal experimentation.[772, 773, 776–778]

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770. E. V. Mertens: Ztschr. f. Krebsforsch. *32*: 82 (1930).

771. F. H. Müller: Ztschr. f. Krebsforsch. *49*: 57 (1939).

772. E. A. Cooper, F. W. M. Lambs, E. Sanders & E. L. Hirst: J. Hyg. *12*: 293 (1932).

773. O. Schürch & A. Winterstein: Ztschr. f. Krebsforsch. *46*: 414 (1937).

774. E. Boyen & R. N. Loomis: Am. J. Cancer *16*: 1515 (1933).

775. A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *8*: 545 (1931); *13*: 287 (1936); Deutsche med. Wchnschr. *63*: 1267 (1937).

776. F. H. Lü: Frankfurt. Ztschr. f. Path. *46*: 513 (1934).

777. K. Sugiura: Am. J. Cancer *38*: 41 (1940).

778. C. M. Flory: Cancer Research *1*: 262 (1941).

Benzpyrene has been isolated from tobacco tar,[779] and an occasional cancer eliciting effect of tobacco and its products in humans cannot be denied, probably requiring an especial susceptibility of the individual. In this respect animal experiments also indicated a promoting action of tobacco tar on the coal tar cancer formation,[768, 776] and in this connection an observation of earlier date may be recalled, according to which painting of normal skin with tobacco tar or also with pyridine resulted in a loss of the carcinolytic activity of extracts prepared from these skin areas.[223]

Supplementing the discussion of organic chemicals with tumorigenic property, brief mention may be made of two substances of rather subordinate significance. One case history has been recorded describing the development of an ulcer on the cheek of a woman following accidental contact with carbon disulfide; a subsequent transformation of this ulcer into an epithelioma was observed.[780] However, this single observation seems insufficient for the establishing of a causal nexus, and no further clinical or experimental material has been presented.

Likewise, a true carcinogenic quality of indole appears problematic. Experiments have been reported in which injection of embryonic pulp together with small amounts of indole caused the development in fowl of rapidly growing sarcomas, whereas embryonic material or indole alone were ineffective;[781, 782] but no confirmation of these results is available. A tumor-favoring action has been ascribed to indole on the basis of the finding that animals, treated for a prolonged period with this substance, exhibited an alteration of their metabolism in a manner which has been considered as characteristic for the tumor process (cf. chapter on metabolism), i.e., a depression of respiration and an augmentation of aerobic glycolysis.[783] These observations might represent an experimental support for the assumption voiced that the accumulation in the intestinal tract of indole and other products of protein putrefaction were to be considered as a factor predisposing to tumor development.[784] The direct application of indole to inoculated tumors, however, has rather been found to cause a growth inhibition,[301, 506] and a similar action was observed from injections of the potassium salt of β-indolacetate, a natural phytohormone,[785] but it appears very likely that these effects depended solely on the great toxicity and the local necrotizing action of these substances. In a recent study, no carcinogenic activity was noted as a result of subcutaneous injections of indole, skatole and heteroauxins (β-(Indole-3)-propionic acid, γ-(Indole-3-n-butyric acid) into mice.[648a]

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[779] A. H. Roffo: Ztschr. f. Krebsforsch. *49*: 588 (1939).

[780] H. Gougerot & Burnier: Bull. Soc. franç. de dermat. et syph. *36*: 1041 (1929); abstr. Cancer Rev. *5*: 642 (1930).

[781] A. Carrel: Compt. rend. Soc. de biol. *93*: 1083 (1925).

[782] M. Askanazy: Wien. klin. Wchnschr. *40*: 609 (1927).

Biological carcinogenic products. This heading comprises a variety of heterogeneous substances derived from biologic substrates and, in contrast to the substances discussed above, not yet defined as to their exact chemical nature. Those experiments might be regarded as most interesting where carcinogenic material has been obtained from tumors or from organs of a tumor-bearing organism. Several investigations of this kind have been performed in recent years with apparent success, the first of which employed benzene extraction of the metastases-free liver of a patient deceased from a gastric cancer. Following injection of this extract to eight mice, malignant tumors (two sarcomas, one carcinoma) arose in three of the animals, and it was held unlikely that these represented merely spontaneous tumors.[786] A detailed account of the technical preparation of the extract employed has been given [787] and, considering the chemical procedures involved, it was not thought probable that carcinogenic substances, not originally present in the liver, had been formed as an artifact during the extraction. Analogous results have been obtained by the same authors in a further study testing a larger experimental material, and it was found that extracts prepared from livers of noncancerous persons also yielded active material.[788] Although one investigator [789] was unable to reproduce these findings, they have been corroborated by the results of experiments, in which painting of the skin of mice with extracts from cancerous and noncancerous Bantu livers resulted in the development of benign and malignant tumors [790] and, in conformance, subcutaneous injections of extracts, derived from European or Bantu livers, caused formation of eleven spindle cell sarcomas among 367 mice treated.[791] In this country also experiments have recently been performed which succeeded in producing thirteen sarcomas in fifty-six mice by injections of benzene extracts obtained from livers of patients who had died of stomach, lung, rectum or esophagus cancer, but this author was unable to elicit such tumors by means of extracts prepared from livers of noncancerous persons.[792] Positive results have also been reported from experiments in which lipid material, extracted from two scirrhous human breast carcinomas, was injected into mice, and elicited a few sarcomas which could be transmitted to other animals.[793] No tumorigenic effect, however, was observed when ether extracts of mammary tissue, derived from female mice of a strain with high-mammary tumor incidence, were injected into mice of another strain.[794] Additional positive results were recently announced: tumorigenic substances, tested in mice, were extracted from human lungs;[795] nonsaponifiable lipid extracts of human livers produced sarcomas upon injection into mice.[796, 796a] With regard to an interpretation of these doubtless interesting results, the elaboration of this problem does not seem to have advanced far enough to permit any suggestions regarding the nature and the origin of the carcinogens derived from tumors or organs of tumor hosts. No experimental support has as yet been given for assuming that these substrates contained any of the defined carcinogens discussed above and, in this connection, it is noteworthy that pertinent investigations, using adequate methods of extraction, have failed to prove the presence of such substances in the urine of cancer patients.[797, 798] However, in a recent communication it has been reported that a carcinogenic fraction could be isolated by the butyl alcohol extraction of urines, previously extracted with benzene; injection of the butyl alcohol fraction into mice resulted, after 16-23 months, in malignant tumors in three out of 34 treated, or out of 16 surviving animals, respectively. One of the tumors arose after administration of cancerous urine extract, whereas the other two were obtained by injections of normal urine preparations. Hence it does not seem established that the urinary excretion of tumorigenic substances is confined to neoplasia but, in view of the scanty experimental material, the authors of this study themselves considered their findings as of a preliminary nature only.[798a] On the other hand, the alternative possibility that a living agent (virus) might be responsible for the tumor development in the experiments mentioned does not seem very likely, in view of the fact that lipid solvents have been used exclusively for the preparation of active extracts, whereas investigations of the filterable tumors of chicken (chapter IX) have conclusively demonstrated that the causative virus possesses protein, and not lipid, character.

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[783] B. Fischer-Wasels: 1st Internat. Cancer Congress Madrid, *2*: 784 (1933).

[784] A. Lorand: Med. Klin. *30*: 1030 (1934).

[785] A. Tanaka & S. Tuboi: Gann *34*: 354 (1940).

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[786] L. Schabad: Compt. rend. Soc. de biol. *124*: 213; *126*: 1180 (1937).

[787] S. A. Neufach: Compt. rend. Soc. de biol. *124*: 616 (1937).

[788] H. E. Kleinenberg, S. A. Neufach & L. M. Schabad: Am. J. Cancer *39*: 463 (1940).

[789] A. Butenandt: Angew. Chem. *53*: 345 (1940).

[790] M. J. A. des Ligneris: Am. J. Cancer *39*: 489 (1940).

[791] I. Hieger: Am. J. Cancer *39*: 496 (1940).

[792] P. E. Steiner: Science *92*: 431 (1940).

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[793] J. F. Menke: Science *92*: 270 (1940).

[794] I. Hieger: Science *93*: 262 (1941).

[795] H. E. Kleinenberg, S. A. Neufach & L. M. Schabad: Cancer Research *1*: 853 (1941).

[796] C. Sannié, R. Truhaut & P. Guérin: Bull. Assoc. franç. p. l'étude du cancer *29*: 106 (1941).

[796a] P. E. Steiner: Am. J. Path. *17*: 667 (1941); Cancer Research *2*: 425 (1942).

[797] H. Sobotka & E. Bloch: Am. J. Cancer *35*: 50 (1939).

[798] R. O. Bowman & H. R. Mottshaw: Cancer Research *1*: 308 (1941).

While the studies just mentioned concerned endogenous carcinogens, another branch of investigation has been directed to the detection of exogenous blastogenic factors, as represented by foodstuffs and their conversion products. In this regard, however, only little convincing material has been presented. A striking announcement has been made as to a blastogenic effect of wheat germ oil. When this product, obtained by ether extraction of wheat germ, was administered perorally to rats, abdominal sarcomas were reported to develop in almost all of the animals, the time required depending on the dosage of the oil and lasting from 15 to 200 days. The tumors exhibited destructive growth and could be transmitted to other animals; rats appeared to be the only species susceptible to the production of these tumors. As far as the chemical nature of the agent contained in wheat germ oil is concerned, it was ascertained only that it was not identical or connected with vitamin E.[799-801] However, an interpreta-

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798a. R. Steele, F. C. Koch & P. E. Steiner: Cancer Research *1*: 614 (1941).

799. L. G. Rowntree, J. Lansbury & A. Steinberg: Proc. Soc. Exper. Biol. & Med. *36*: 424 (1937).

800. G. M. Dorrance & E. P. Ciccone: Proc. Soc. Exper. Biol. & Med. *36*: 426 (1937).

801. L. G. Rowntree, A. Steinberg, G. M. Dorrance & E. P. Ciccone: Am. J. Cancer *31*: 359 (1937).

tion and evaluation of these interesting communications does not appear feasible, since a great number of authors failed completely in their attempts to corroborate these findings,[802-812a] which indicates possibly that some unknown, decisive factor or factors were involved in the original experiments and escaped reproduction in the later investigations. The fact that tar preparations with carcinogenic activity for animals could be derived from coffee[813] and from tea leaves,[814] is of theoretical interest only according to the view of the author himself, since these tarry products are water-insoluble and therefore not contained in the foods consumed. In addition, it appears more than doubtful whether the customary preparations of these foods bear any analogy to the chemical procedures involved in the laboratory experiments, and most likely similar tar preparations could be derived by chemical methods from almost any vegetable or animal substrate. A closer connection to actual food processing seems to be exhibited by experiments using ether extracts of roasted

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802. C. Dittmar & C. Burschkies: Ztschr. f. Krebsforsch. *49*: 379 (1939).

803. C. Carruthers: Proc. Soc. Exper. Biol. & Med. *40*: 107 (1939).

804. C. R. Halter: Proc. Soc. Exper Biol. & Med. *40*: 257 (1939).

805. H. M. Evans & G. A. Emerson: Proc. Soc. Exper. Biol. & Med. *41*: 318 (1939).

806. E. Dingemanse & N. F. van Eck: Proc. Soc. Exper. Biol. & Med. *41*: 622 (1939).

807. R. Auchincloss & C. D. Haagensen: Proc. Soc. Exper. Biol. & Med. *42*: 703 (1939).

808. C. Sannié & R. Truhaut: Compt. rend. Soc. de biol. *131*: 600 (1939).

809. L. L. Ginzton & C. L. Connor: Am. J. Cancer *38*: 90 (1940).

810. D. L. Rider: Am. J. Cancer *38*: 275 (1940).

811. H. Blumberg: Pub. Health Rep. *55*: 53 (1940).

812. H. P. Rusch, C. A. Baumann & G. L. Maison: Arch. Path. *29*: 8 (1940).

812a. A. M. Brues, B. B. Marble & B. Riegel: Cancer Research *1*: 815 (1941).

813. A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *15*: 741 (1938).

814. A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat. d. cancer *19*: 661 (1940).

foodstuffs (coffee, horse muscle, browned butter) which were tested by painting the skin of mice; nine tumors arose among twenty-three treated female mice, while only one tumor was observed in twenty-six control animals. Considering the small scale of the experiments, the author conceded that they could not yet be regarded as conclusive.[815]

C. Organic Chemotherapy of Tumors

The term chemotherapy is used in the following, but not in the classic sense (Ehrlich), implying a specific adjustment between the therapeutic agent and the diseased substrate, as has been possible to accomplish in certain infectious diseases. As far as neoplasia is concerned, curative measures of this character have up to now been non-existent, because of the failure of all efforts to create the required theoretical basis for their realization. Therefore the term chemotherapy is used here with a broader meaning, that is, the chemotherapeutic attacks on the cancerous process have principally been based on endeavors to depress the rate of the malignant growth, to interfere with the metabolism of the neoplasms as far as it has been recognized to deviate from that of normal tissue, and, generally, to create a milieu which might be presumed to be unfavorable for the development or the advancement of malignant tumors. Interpretations of this kind must be applied to the majority of the cancer curative proposals enumerated in the following. Specific therapeutic measures could not be devised, and may hardly be expected.

Alcohols. The results of numerous investigations on the influence of ethyl alcohol on animal tumors may be summarized, in that the peroral ingestion of even large doses was followed only by very slight growth inhibitions,[506, 816–819]

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815. E. M. P. Widmark: Nature, London *143*: 984 (1939).
816. A. Wrzosek: Ztschr. f. Krebsforsch. *11*: 515 (1912).
817. O. Macchiarulo: Ztschr. f. Krebsforsch. *26*: 15 (1928).

whereas subcutaneous[820] or intravenous[821] administration resulted in marked growth inhibition of the animal tumors. The latter procedure effected cures in sixty per cent of rat sarcomas when the animals received intravenous injections of 1 c. c. of absolute alcohol, certainly an extraordinary undertaking, which, however, did not affect rat carcinomas. Intravenous injections of small doses of alcohol to rabbits with Kato sarcoma accelerated, whereas large amounts inhibited, the tumor growth.[822] The use of alcohol injections in human cancer pathology yielded beneficial results in only a few cases[823] or subjective improvement.[824] The peritumoral alcohol injection was found to alleviate pain, the first injection itself being painful, but not the following ones and, in some instances, growth cessation seemed to follow the treatment.[825] The local application of amyl alcohol in ulcerated skin cancer has been claimed to result in diminished secretion, and sometimes in reduction of the tumor size,[826] but it appears doubtful whether this expressed more than merely an antiseptic or antiphlogistic effect.

Aliphatic aldehydes. Formaldehyde (formalin) has been applied externally, both in animal experiments and in clinical cases, and consecutive necrosis, induration and insensitivity of the tumor masses were observed but no permanent growth

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818. M. C. Marsh: Am. J. Cancer *19*: 847 (1933).

819. U. Suzuki, W. Nakahara, N. Hashimoto & R. Ikeda: Scient. Paper Inst. Phys. & Chem. Res. Tokyo *23*: 9 (1933); abstr. Am. J. Cancer *22*: 414 (1933).

820. Tsurumi: Ann. Inst. Pasteur *30*: 346 (1916).

821. D. Thursz: Ztschr. f. Krebsforsch. *25*: 323 (1927).

822. H. Yasunami: Jap. J. Obst. & Gynec. *23*: 214 (1940).

823. D. Thursz: Ztschr. f. Krebsforsch. *26*: 260 (1928); Polski Przegl. chir. *12*: 660 (1933); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 28 (1935).

824. Manceaux, Fangeaux & Boukeloua: Algérie méd. *38*: 261 (1934); abstr. Am. J. Cancer *24*: 705 (1935).

825. D. Férey: Presse méd. *40*: 1125 (1932).

826. Horand: Jour. de Prat. 1910, No. 10; abstr. Wien. klin. Wchnschr. *23*: 1573 (1910).

cessation could be achieved;[827] the effects mentioned depended obviously on a protein coagulation caused by formalin. Formalin applications have been recommended in uterine cancer also,[828] and it was claimed that following this treatment some inoperable tumors became operable,[829] probably in consequence of subsiding inflammation. The favorable results obtained by the treatment of roentgen-irradiated skin carcinomas with concentrated formalin solutions[830] most likely depended to a small extent only on the aldehyde. The intratumoral injection of a one per cent formalin solution caused regressions of Jensen rat sarcomas, the cured animals being immune against a repeated tumor inoculation,[831] but the technique employed cannot be considered as convincing, and subcutaneous injections of formaldehyde sulfoxylate proved ineffective in the Walker rat carcinoma.[832] Diformaldehyde diperoxide was claimed to inhibit the tumor formation in mice treated with benzpyrene[833, 834] and further studies showed that this inhibitory action could be achieved with small doses only, while larger amounts of the peroxide enhanced the tumor development.[835, 836] However, extensive experiments recently undertaken with the compound in question (designated as di-(hydroxymethyl)-peroxide) yielded completely negative results; no effects were discernible when the substance was administered to mice bearing transplanted tumors, spontaneous tumors or tumors induced by methylcholanthrene.[836a]

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[827] A. F. W. Powell: Brit. M J. *1*: 1237 (1903).

[828] A. Ranelletti: Semaine méd. 1900, No. 1; abstr. Berlin. klin. Wchnschr. *37*: 31 (1900).

[829] V. Czerny: Arch. f. klin. Chir. *61*: 287 (1900).

[830] H. Laurell: Upsala Läk. for Förh. *40*: 169 (1934); abstr. Ztschr. f. Krebsforsch. *43*: Ref. 138 (1936).

[831] T. Lumsden: Lancet *2*: 1283 (1927).

[832] J. C. Krantz, Jr., R. Musser, C. J. Carr & W. G. Harne: Am. J. Cancer *25*: 789 (1935).

[833] J. Maisin: Compt. rend. Soc. de biol. *120*: 106 (1935).

[834] J. Maisin & H. Vassiliadis: Libro de Oro, Buenos Aires, p. 799 (1935).

[835] J. Maisin & F. Robert: Compt. rend. Soc. de biol. *123*: 156 (1936).

[836] J. Maisin, Y. Pourbaix & P. Caeymaex: Compt. rend. Soc. de biol. *127*: 1479 (1938).

Glyceric aldehyde has been included in therapeutic experiments because of its ability to inhibit tumor glycolysis;[837] yet, in spite of this, it failed to influence animal tumors beneficially.[838] Propionic aldehyde has been found to exert a reversible, non-toxic inhibition of the mesenchyme,[839] and correspondingly has been injected subcutaneously into mice treated with benzpyrene, with the result that the tumor yield was reduced to twenty-two per cent from thirty-six per cent in control animals;[840] this effect must be considered as a slight inhibition only, and is in need of further investigation.

Heptylaldehyde has been the subject of numerous studies which were started by the observation that oil of wintergreen (gaultheria), fed daily to mice of a strain with high mammary cancer incidence, delayed the tumor development and decreased the total tumor incidence.[841] In further work, this preventive action of the plant oil was confirmed and gaultheria oil also effected complete cure in four already developed tumors out of thirty-four treated animals. The active substance was recognized as being contained in the low-boiling fraction of the oil[842] and was identified as heptylaldehyde, the feeding of which substance to twenty-five mice with spontaneous breast cancer resulted in softening of the tumors and in complete regression of six neoplasms.[843] Favorable results were reported also from heptylaldehyde injections into dogs bearing spontaneous tumors.[844] Subcutaneous injections of heptylaldehyde sodium bisulfate caused regression of spontaneous mammary cancer in ten per cent of the treated mice and suppressed the incidence of lung metastases, provided adequately large doses were administered.[845] A re-examination confirmed the inhibition of spontaneous mouse tumors by means of heptylaldehyde and, among other aldehydes and ketones tested, citral was found to act similarly. But these effects were limited to spontaneous tumors and to tumors induced by methylcholanthrene whereas inoculated tumors (Crocker mouse sarcoma) were not influenced.[846, 847] A further corroboration of the heptylaldehyde action on spontaneous tumors has been given,[848] while the growth of inoculated rat sarcomas was not found inhibited.[849, 850] The alternating, external application of methylcholanthrene and heptylaldehyde inhibited the tumor formation and the cancerization of papillomas in mice, while developed tumors were not affected; it was assumed that an inhibition of glycolysis might explain this effect of heptylaldehyde.[851] Other workers, however, obtained divergent results, insofar as neither spontaneous, nor induced or inoculated tumors showed signs of marked growth inhibition following heptylaldehyde administration,[852, 853] and slight inhibitions, occurring when very large doses of the aldehyde were used, have been considered as a consequence only of the reduced caloric intake, due to the toxic effect of the drug.[852] Furthermore, it has been reported that heptylaldehyde injections into mice with spontaneous tumors exerted a destructive action on kidneys and liver, far outweighing the observed inhibition of tumor growth, which therefore might be regarded merely as an expression of a general toxic action.[854] Hence it may be concluded that certain aliphatic aldehydes, under suitable experimental conditions, inhibit the growth of some animal tumors, by means of an interference with the metabolism—peroxide formation, oxygen liberation, depression of glycolysis—but it is doubtful whether this action is directed specifically against the neoplastic cells, or whether rather it does not lead to a debilitated general health of the animals, thus causing an indirect inhibition of the tumor growth, due to impaired nutrition. Similar relations may be involved in experiments using the substituted ketone, viz. monochloracetone, a specific inhibitor of cell glycolysis, which impeded the tumor formation by benzpyrene in mice, if low concentrations of the ketone were administered, whereas higher concentrations were ineffective, and very high ones even slightly stimulated the tumor development.[855]

[836a] M. Belkin: Cancer Research *2*: 264; 269; 276 (1942).

[837] B. Mendel: Klin. Wchnschr. *8*: 169 (1929); *9*: 118 (1930).

[838] B. Fischer-Wasels: Gastherapie bösartiger Geschwülste, Frankf. a. Main, 1929, p. 93.

[839] C. M. Pomeret & E. N. Willmer: J. Exper. Biol. *16*: 232 (1939).

[840] J. F. Riley & A. B. Wallace: Brit. J. Exper. Path. *22*: 24 (1940).

[841] L. C. Strong: Am. J. Cancer *20*: 387 (1934); *23*: 297; *25*: 797 (1935); Am. J. M. Sc. *192*: 546 (1936).

[842] L. C. Strong: Am. J. Cancer *32*: 227 (1938).

[843] L. C. Strong: Science *87*: 144 (1938); Am. J. Cancer *35*: 401 (1939).

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[844] L. C. Strong & L. F. Whitney: Science *88*: 111 (1938).

[845] L. C. Strong: Proc. Soc. Exper. Biol. & Med. *43*: 634 (1940); Cancer Res. *1*: 473 (1941).

[846] E. Boyland & E. H. Mawson: Biochem. J. *32*: 1982 (1938).

[847] E. Boyland: Biochem. J. *34*: 1196 (1940).

[848] C. Dittmar: Ztschr. f. Krebsforsch. *49*: 515 (1939).

[849] W. G. Clark: Proc. Soc. Exper. Biol. & Med. *40*: 562 (1939).

[850] C. Carruthers: Proc. Soc. Exper. Biol. & Med. *44*: 41 (1940).

[851] C. Carruthers: Arch. Path. *30*: 1184 (1940).

[852] C. A. Baumann, B. E. Kline & H. P. Rusch: Proc. Soc. Exper. Biol. & Med. *39*: 354 (1939).

[853] M. L. Long & F. Bischoff: Am. J. Cancer *38*: 406 (1940).

Aliphatic acids and their halogen derivatives. Only a few investigations have dealt with aliphatic acids. In some of them formic acid was employed in clinical experiments, but only few cancer cases were treated, and these with uncertain results.[856] No greater significance can be assigned to a claim that injections of homeopathic doses of formic acid benefited cancer patients.[857] Ethyl chaulmoograte, an ester of an unsat-

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854. F. Garai: Cancer Research *1*: 144 (1941).

855. H. G. Crabtree: Cancer Research *1*; 34 (1941).

856. Krull (1906); Evans (1908); quot. C. Lewin: Ergebn. d. inn. Med. u. Kinderh. *8*: 499 (1912).

857. A. Reuter: Ameisensäure als Heilmittel; München 1936, Verl. d. ärztl. Rundschau Otto Gmelin.

urated plant acid, used in the treatment of leprosy, has been injected into rats bearing transplanted sarcomas, with the result that twelve tumors regressed among 120 treated animals, whereas tumors were never observed to recede in control animals or in those treated with a great number of other aliphatic acids or their esters, including chaulmoogric acid.[858] As to therapeutic investigations undertaken with butyric acid and lactic acid, reference is made to the first section of this chapter.

More attention has been devoted to halogen-substituted aliphatic acids, since it was found that monoiodo-acetic acid and monobromacetic acid selectively suppressed the glycolysis of biologic substrates.[859] Investigations of tissue cultures, furthermore, indicated that neoplastic cells were more sensitive toward these compounds, than normal ones, and inoculation of tumor material to which the halogen acids had been added did not lead to tumor development, thus proving their loss of vitality.[860] But injections of monoiodo-acetate or monobromacetate failed to influence the growth of transplanted tumors in most experiments,[861-863] and negative results were likewise obtained with monobromcaproic acid [864] of which substance a greater effect had been expected because of a better diffusibility. Ethyl bromacetate, tested on the ascites carcinoma of mice, was also ineffective.[865] More favorable effects have been reported when subcutaneous in-

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858. J. A. Pollia: 3rd Internat. Cancer Congress, Atlantic City, p. 133 (1939).
859. E. Lundsgaard: Biochem. Ztschr. *220*: 8 (1930).
860. A. A. Krontowski, M. K. Jazimirska-Krontowska & H. P. Ssawitzka: Ztschr. f. Krebsforsch. *37*: 457 (1932).
861. A. A. Krontowski, M. A. Magath & E. J. Smailowskaja: Ztschr. f. Krebsforsch. *38*: 495 (1932); Vopr. Onkol. *6*: 55 (1934).
862. J. J. Jares: Am. J. Cancer *23*: 289 (1935).
863. F. Wyss-Chodat: Bull. Schweiz. Verigg. Krebsbek. *3*: 98 (1936).
864. W. A. Selle & M. Bodansky: Am. J. Cancer *23*: 289 (1935).
865. H. Henkel: Ztschr. f. Krebsforsch. *51*: 199 (1941).

jections of not neutralized monoiodo-acetic acid were combined with the peroral administration of acidifying salts, such as ammonium chloride or calcium chloride; this procedure always resulted in a reduction of the size of transplanted mouse and rat tumors, and in approximately twenty per cent of the animals complete tumor regression was observed.[866] In other animal experiments, beneficial effects were obtained by combining injections of iodo-acetic acid with roentgen radiation,[867] and in concurrence with these findings, the injection of monoiodo-acetic acid was seen to heighten the radio-sensitivity of mouse sarcoma.[868] It has, however, been emphasized that the therapeutic use of bromacetic and iodoacetic acid was inadvisable because of their high toxicity, and investigation of monobromsuccinic and monobrommalonic acid was recommended, since these compounds were found to be less toxic.[869]

Prussic acid and its salts. Investigations on these extremely toxic substances have only theoretical interest, particularly since animal experiments demonstrated the necessity of using sublethal doses for purposes of tumor inhibition. Thus, injection of potassium cyanide in doses which provoked convulsions effected complete regression of fourteen out of ninety-eight treated Ehrlich mouse carcinomas,[870] and an inhibition of transplanted tumors was also observed when the animals were exposed to inhalation of prussic acid.[871] The latter treatment resulted sometimes in cure of the tumors, and the transplantability of treated tumors was regularly

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866. A. Goldfeder: Ztschr. f. Krebsforsch. *39*: 417; 421 (1933).

867. G. Harkert & W. Moppett: Austral. J. Exper. Biol. & Med. *14*: 15 (1936).

868. W. R. Franks, M. M. Shaw & W. H. Dickson: Am. J. Cancer *22*: 601 (1934).

869. C. Dittmar: Ztschr. f. Krebsforsch. *49*: 515 (1939).

870. L. Karczag & M. Csaba: Med. Klin. *23*: 1413 (1927).

871. L. C. Maxwell & F. Bischoff: J. Pharmacol. & Exper. Ther. *49*: 270 (1933).

found markedly impaired, but in all these experiments the effective dose of prussic acid came very close to the lethal one.[872] The toxic action of prussic acid was also evidenced by vitro experiments in which M/500 to M/1000 solutions of potassium cyanide were added to tumor slices and as a consequence a decrease of 85–95 per cent of the respiration was observed, simultaneously with an increase of the radiosensitivity.[873] An analogous behavior of the radiosensitivity was noted in vivo, when potassium cyanide was injected into sarcoma-bearing mice.[867] Experiments, however, performed with the Walker rat sarcoma [319] failed to indicate any effect on the tumor, even of very high doses of potassium cyanide.[874] Generally, it may be assumed that a tumor inhibiting effect of cyanides, when apparent, must be due to a severe respiratory disturbance, affecting, in favorably responding cases, the tumor more than the normal tissue. Such a view seems supported by observations that another respiratory poison, carbon monoxide, exerted similar effects upon tumor growth.[871, 875]

Lipid solvents. Several simple aliphatic compounds such as ethyl ether, chloroform and carbon tetrachloride have been tested as to their influence on animal tumors, because of their common physical character as lipid solvents and narcotics, which should make it possible for them to penetrate the cell walls, and in turn to affect the cell permeability, but injections of saturated solutions of these substances in horse serum failed to evoke any significant alteration of the tumors.[876] Other authors, however, noticed that chloroform

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872. I. H. Perry: Am. J. Cancer *25*: 592 (1935).

873. H. G. Crabtree & W. Cramer: Proc. Roy. Soc. London, s. B *113*: 226; 238 (1932).

874. J. C. Krantz, Jr., R. Musser, C. J. Carr & W. G. Harne: Am. J. Cancer *27*: 293 (1936).

875. J. A. Campbell: J. Path. & Bact. *35*: 379 (1932).

876. M. J. Shear: Am. J. Cancer *25*: 66 (1935).

injections, administered previous to the implantation of Fujinawa rat sarcoma, retarded the subsequent tumor growth,[508] whereas the growth of Kato rabbit sarcoma was reported to be accelerated when the animals received intravenous injections of not too large doses of ether or chloroform.[822] An investigation of ethylene, (C_2H_4), an unsaturated aliphatic hydrocarbon, has been stimulated by the observation that this gas retarded tissue regeneration, and impeded the growth of normal and neoplastic tissue cultures; an analogous effect became apparent when tumor transplants were exposed to ethylene gas prior to their inoculation. The latent period of tumor development was lengthened, the total tumor yield diminished (44 per cent, as compared with 74 per cent in controls), and the growth of the remaining tumors was delayed. Local ethylene application, starting soon after the inoculation, also decreased the number of takes (twenty-two per cent, as compared with ninety-six per cent in controls), and exposure of large tumors to the gas resulted, in fifteen per cent of the animals, in regression of the tumors whereas the others showed a markedly diminished growth rate.[877] These studies, however, were performed with a transplanted tumor of unusual character (myxosarcoma of rats, originally obtained by Kritschewski and Sinelnikow by heterotransplantation of a human melanosarcoma to rats), and confirmations of these observations have not been forthcoming.

Lead tetraethyl, a substance used in internal combustion engines to prevent "knocking," has been employed in mice with spontaneous carcinomas, leading to tumor involutions when sublethal doses were administered.[878] But since this compound exhibits an extreme toxicity, and accordingly the treated animals showed signs of severe health impairment,

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877. K. A. Friede & M. K. Ebert: Ztschr. f. Krebsforsch. *40*: 431 (1934).

878. M. C. Marsh & B. T. Simpson: J. Cancer Res. *11*: 417 (1927).

neither theoretical nor practical conclusions may be inferred from these experiments.

The observation that addition of ββ′-dichlordiethylsulfide (mustard gas) suppressed the carcinogenic activity of coal tar [879-881] does not seem to have any cancer curative aspect, since the mechanism of this phenomenon was recognized as involving a damage to the skin, thus preventing the formation of the precancerous papillomas. But if benign warts had already developed, mustard gas did not prevent their conversion into malignant tumors. Cantharidine, another vesicant, was found to exert a similar inhibitory effect on the tar cancer induction,[882] but it will be recalled that production of acute inflammation alone has been found without influence on carcinogenesis.[659] Furthermore, it has been noticed that mustard gas and cantharidine, which in vivo inhibited the tar cancer formation, in vitro lowered the glycolysis of Jensen rat sarcoma more strongly than the respiration, whereas chemically related substances, which had no effect on the tar cancer development, failed to interfere with the tumor metabolism.[883] Nevertheless, it has been maintained that the tar cancer inhibition observed is due only to a local action of these substances, not involving a true inactivation of the tar, or a systemic alteration in the animals treated, and clinical application of mustard gas in three skin cancers failed to show any results.[884]

Choline. This organic base, trimethyl-hydroxyethyl-ammoniumhydroxide, is a constituent of the lecithin molecule,

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879. I. Berenblum: J. Path. & Bact. *32*: 425 (1929).

880. J. Deloof: Cancer, Bruxelles *8*: 89 (1931).

881. J. Visser & J. J. Th. Vos: Geneesk. tijdschr. v. Nederl.-Indië *75*: 1363 (1935); abstr. Ztschr. f. Krebsforsch. *43*: Ref. 157 (1936).

882. I. Berenblum: J. Path. & Bact. *40*: 549 (1935).

883. I. Berenblum, L. P. Kendal & J. W. Orr: Biochem. J. *30*: 709 (1936).

884. J. Visser & R. E. J. Ten Seldam: Geneesk. tijdschr. v. Nederl.-Indië *77*: 3092 (1937).

and has been held responsible for some curative effects achieved with lecithin, as mentioned above.[409] An observation of older date, that roentgen radiation causes the cleavage of choline from lecithin,[885] led to the theory that this phenomenon should represent the effective biologic factor in the response to irradiation,[886] a view which seemed supported by the fact that choline injections elicited a dermatitis of similar appearance to the skin response to X-rays. Although modern radiobiology cannot accept this attempt at explanation of the radiation effects, based on one simple chemical alteration, at that time this theory gave rise to experimental work using choline in neoplastic conditions. Mouse tumors were found to recede following injections of choline, but no prolongation of life was observed on account of the high toxicity of this substance. Better results were achieved in animal experiments if salts of choline with iodobenzoic acid or glycine were used.[887] For clinical purposes a preparation, called "enzytol" and consisting mainly of choline borate, has been preferred. It was claimed that intravenous injections of this substance brought about remarkable improvements in about twenty per cent of 323 inoperable cancer patients treated, some of whom seemed clinically healed.[888] Other favorable experiences with this treatment were reported by several clinicians,[889-891] and combination of roentgen and choline therapy has been proposed.[892] However, other workers [893, 876] found choline administration to be ineffective in animal experiments, and the clinical choline therapy soon became obso-

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885. G. Schwarz: Pflüger's Arch. *100*: 532 (1903).
886. R. Werner: Strahlentherapie *1*: 442 (1912).
887. R. Werner & L. Ascher: Strahlentherapie *1*: 452 (1912).
888. Rapp: München. med. Wchnschr. *61*: 1112 (1914).
889. G. Klein: München. med. Wchnschr. *61*: 115 (1914).
890. Ch. Vermeulen: abstr. Deutsche med. Wchnschr. *40*: 452 (1914).
891. C. Lewin: Ztschr. f. Krebsforsch. *32*: 128 (1930).
892. R. Werner: Berl. klin. Wchnschr. *50*: 435 (1913).
893. S. Fränkel & E. Fürer: Wien. klin. Wchnschr. *29*: 96 (1916).

lete in spite of the initial successes, which obviously could not withstand a more critical evaluation. Therefore it is only of historical interest to refer to a case history of a patient suffering from uterine carcinoma, in whom a growth cessation of the tumor for four years occurred following eating of poisonous choline-containing mushrooms,[894] or to refer to an explanation which attributed the beneficial effect sometimes observed from injections of tumor autolysates to their choline content.[895] A preparation, "cancroin," designated as neurine solution, was recommended at the end of the last century as a cancer cure,[896] but was recognized as actually containing choline,[893] and the far reaching claims advanced as to its cancer curative effect [896, 897] have been completely rejected by experienced clinicians.[898-901]

Another organic base containing nitrogen, synthaline (dodekamethylenguanidine), which had acquired a transitory interest with relation to diabetes by virtue of its ability to produce hypoglycemia, has been applied locally in human skin cancer, on account of its supposedly similar action to that of insulin (cf. chapter on hormones), and it was claimed that beneficial results had been achieved by this treatment.[902] Transplanted animal tumors, however, reacted with a slight growth inhibition only, attributable to the general health impairment, when synthaline was administered.[903] Similar ob-

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894. E. Opitz: Strahlentherapie *3*: 251 (1913).

895. J. Sellei: Z. f. Chemother. *1* (1913); abstr. Ztschr. f. Krebsforsch. *13*: 398 (1913).

896. A. Adamkiewicz: Untersuchunger über den Krebs, Wien 1893, Braumüller & Sohn; Berl. klin. Wchnschr. *39*: 569 (1902).

897. L. Kugel: Berl. klin. Wchnschr. *39*: 567 (1902).

898. H. Nothnagel: Berl. klin. Wchnschr. *39*: 659 (1902).

899. A. Eiselsberg: Berl. klin. Wchnschr. *39*: 659 (1902).

900. Poten: Berl. klin. Wchnschr. *39*: 660 (1902).

901. Schultz-Schultzenstein: Berl. klin. Wchnschr. *39*: 661 (1902).

902. F. S. Gomes da Costa: Ztschr. f. Krebsforsch. *39*: 5 (1935).

903. F. Bischoff & L. C. Maxwell: J. Pharmacol. & Exper. Ther. *42*: 387 (1931).

servations were made on spontaneous mouse tumors which, furthermore, resumed a normal growth rate as soon as medication was stopped.[904]

Benzene and substitution products. The basic compound of the aromatic series, benzene, attracted attention because of its ability to reduce the number of leucocytes, as is known both from occupational diseases in benzene plant workers, and from observations in leukemia. As far as malignant neoplasms are concerned, intratumoral injections of benzene have been tried in cancer patients, but this extremely painful procedure resulted in only local necrosis, while the periphery of the neoplasm showed unabated growth.[905] Peroral administration of benzene to sarcoma-bearing rats has been reported as inhibiting growth of the tumors,[906] and benzene injections retarded the development of the Kato rabbit sarcoma, at the same time, however, decreasing the radiosensitivity of the tumors.[907] It was assumed that the leucocyte drop, accompanying the benzene treatment, might be responsible for the tumor inhibition inasfar as decomposition products liberated from the destroyed white blood cells would stimulate the defense mechanism of the body,[907] but, on the other hand, the tumor inhibition resulting from benzene administration, very likely might merely be the expression of a general toxic effect of this substance.

Benzaldehyde, salicylic aldehyde, thymol and caryacrol were found to impede the growth of inoculated tumors, only if brought into direct contact with the transmitted material.[908] Similar results have been obtained with phenol, tricresol and zephirol.[909] It is almost certain that these effects represent only an unspecific cell damage, although in tissue cultures the growth of fibroblasts was less affected by these substances than the proliferation of neoplastic cells.[909] A beneficial response ascribed to the use of salicylic acid in skin cancer [910] obviously must be interpreted as an uncharacteristic caustic reaction and, likewise, guaiacol and its derivatives cannot be regarded as exhibiting a cancer curative quality, notwithstanding the fact that their clinical use has been recommended for the alleviation of pericancerous inflammation and for the relief of pain.[911] The testing of almost one hundred different halogen-substituted aromatic compounds, as to their influence on animal tumors, demonstrated a growth inhibiting action of tetrabromocresols only, but a clinical use of this substance seems out of the question because of the severe pains following its injection.[912] The tumor formation in mice which were painted with dibenzanthracene has been reported as being impeded by simultaneous or alternate application of p-thiocresol and of the hydrocarbon, for which effect the sulfhydryl group of this compound was held responsible.[913]

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[904] E. Boyland: Biochem. J. *32*: 1207 (1938).
[905] G. Királyfi: Berl. klin. Wchnschr. *50*: 1982 (1913).
[906] R. Somogyi: Ztschr. f. Krebsforsch. *17*: 100 (1920).
[907] S. Sasaoka: Jap. J. Obst. & Gynec. *22*: 86; 90 (1939).
[908] N. Waterman: Ergebn. d. inn. Med. u. Kinderh. *30*: 304 (1926).
[909] B. Lustig & E. Werber: Ztschr. f. Krebsforsch. *43*: 364 (1936).

A broader theoretical foundation appeared to be available for investigations of nitrobenzene derivatives, particularly of dinitrophenols, since it has been established that these compounds, which are known to increase the metabolism rate energetically and to cause hyperthermia, also inhibited cell mitosis,[914] influenced the tumor metabolism in vitro [915] (cf. chapter on metabolism), and increased the radiosensitivity of tissues.[916] Based on these facts, the possibility of a thera-

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910. C. Weinbrenner: München. med. Wchnschr. *61*: 127 (1914).

911. A. Neumann: Wien. med. Wchnschr. *80*: 859 (1930); 1st Internat. Cancer Congress Madrid, *2*: 1087 (1933).

912. H. R. Gaylord: Berl. klin. Wchnschr. *49*: 2017 (1912).

913. S. P. Reimann & E. M. Hall: Arch. Path. *22*: 55 (1936).

914. M. E. Krahl & G. H. A. Clowes: Proc. Soc. Exper. Biol. & Med. *32*: 226 (1934).

915. E. C. Dodds & G. D. Greville: Lancet *1*: 398 (1934).

916. J. Firket: Arch. de biol. *46*: 507 (1935).

peutical use of these substances in neoplasia has been implied,[917] but experimental studies demonstrated the ineffectiveness of this measure. Peroral or parenteral administration of dinitrophenols did not impede the growth of rat tumors, even if doses were employed causing a temperature increase to 42° C,[918] and sublethal quantities of this substance failed to affect mouse sarcoma 180, in spite of a considerable loss of total body weight occurring in the animals treated.[919] Likewise, dinitrophenol did not influence the tar cancer formation in mice,[920] and feeding of dinitro-o-cresol to mice, treated with tar, even resulted in an earlier appearance of malignant tumors.[921] Similarly, tumor induction by means of benzpyrene was promoted if the animals received dinitrophenol injections, and it has been assumed that the increase of the metabolism, brought about by this treatment, would favor the development of neoplasms.[922] Picric acid, a trinitrophenol, has been found to be without effect on inoculated mouse tumors.[923]

Aromatic compounds containing sulfur, such as sulfonamide, prontosil, etc., which assumed a great significance in the treatment of infectious diseases, were reported to exert only a temporary growth inhibition of spontaneous and transplanted mouse tumors; in this respect, pp′-diaminodiphenylsulfoxide and sodium sulfanilyl-sulfanilate were noticed as being the most effective.[904]

Organic dyes. Endeavors to utilize these substances for tumor therapy are going back to the idea, conceived by Ehrlich, that it might be possible to destroy tumor cells by means of chemicals which exhibit a great affinity for them, such as seemed the case with some dyes which intensively stained neoplastic tissue. At least, it was reasoned, these organic dyestuffs could serve as vehicles transporting other tumor-destroying agents to the cells, and, with this goal in mind, Wassermann and his collaborators[924] proposed, and experimented with, a selenium-eosin compound, as has been discussed in the first chapter. It will, however, be demonstrated in the next chapter, that the presupposed selective affinity of dyestuffs to tumor cells has not been proven for any of the substances tested, but that the stains were principally localized in the necrotic parts, or in the connective tissue of the neoplasms. This circumstance makes it comprehensible that the pertinent investigations, testing a great number of compounds, led to regrettably unsatisfactory results. In one study, not less than 144 various aniline dyes were tried in spontaneous mouse carcinomas, without any recognizable therapeutic effect,[925] and, clinically, methyl violet,[926] trypan red,[927] trypan blue,[928] trypaflavine and its salts,[929, 930] have been used only with very insignificant results. Thirty-four basic dyes derived from xanthene, acridine, quinoline and other compounds, were found to inhibit the growth of tissue cultures of sarcoma, but also of spleen,[931] and since normal and neoplastic growth were influenced in the same way, it is doubtful whether this finding could acquire practical significance. In this connection a report may be mentioned, according to which treatment of mouse tumors with the cadmium salt of acridinium nitrate resulted in a cure of some of the animals.[932] Trypan blue injections, both locally administered or far from the tumor site, were seen to increase the radiosensitivity of rat sarcomas,[933] but the use of this dye seems inadvisable on account of its damaging effect on the reticulo-endothelial system and a resulting promotion of the cancerous process (cf. section on reticulo-endothelial system).

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[917] H. Handovsky: Wien. klin. Wchnschr. *49*: 1301 (1936).

[918] L. A. Emge, L. M. R. Wulff & M. L. Tainter: Proc. Soc. Exper. Biol. & Med. *31*: 152 (1933).

[919] F. Bischoff & M. L. Long: Am. J. Cancer *31*: 67 (1937).

[920] K. A. Vannfält: Upsala Läk. for Förh. *42*: 55 (1936); abstr. Ztschr. f. Krebsforsch. *45*: Ref. 95 (1937).

[921] L. Kreyberg: Am. J. Cancer *36*: 51 (1939).

[922] P. Giliberti: Tumori n. s. *14*: 222 (1940).

[923] Ch. André: Libro de Oro, Buenos Aires, p. 16 (1935).

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[924] A. Wassermann, F. Keysser & M. Wassermann: Deutsche med. Wchnschr. *37*: 2389 (1911).

[925] B. T. Simpson: Internat. Conf. Cancer, London, p. 208 (1928).

[926] R. von Mosetig-Moorhof: Zentralbl. f. d. ges. Therap. (1896); quot. 856.

[927] G. Fichera: Chimioterapia del Cancro, Ulrico Hoepli, Milano 1934.

[928] E. Opitz, K. Vorländer & H. Jung: Munchen. med. Wchnschr. *73*: 1624 (1926).

[929] C. Lewin: Ztschr. f. Krebsforsch. *32*: 176 (1932).

[930] F. Blumenthal: Beitr. z. klin. Chir. *154*: 50 (1932).

[931] K. Tokumitsu: Tr. Soc. path. jap. *26*: 701 (1936).

Far reaching claims have been advanced as to an alleged cancer curative quality of isamine blue.[934-936] Injections of this dye were reported to have caused the regression of transplanted mouse tumors, whereas spontaneous tumors reacted less favorably, and tar carcinomas not at all.[937] Isamine blue treatment was also claimed to have effected cures of a considerable percentage of rat sarcomas, rat carcinomas and rabbit sarcomas,[938] or growth inhibition of rabbit sarcoma by using intravenous or subcutaneous injections,[939] or inhibition of rat carcinoma in which latter experiments peritumoral injections of isamine blue were administered.[940] Other authors, however, observed no effect of isamine blue on transplanted mouse tumors,[928, 941] and, similarly, the favorable experiences reported as being obtained with isamine blue in cancer patients,[934, 936] sometimes in combination with diathermy,[942] have not been confirmed by other workers, inasmuch as symptomatic relief only was seen,[943] or even untoward consequences of the treatment, such as collapse, were observed.[944, 945] How misleading reports of alleged clinical cancer cures may be has been evidenced by an autopsy of a case in which the "cure" of a cancer of the colon had been achieved, it was claimed, by means of isamine blue therapy, whereas the post mortem examination revealed the condition as a chronic colitis.[946] In view of the doubtful practical value of the isamine blue treatment, it seems of little importance only whether the mechanism involved is connected with a dehydration of the tumors,[947] or whether isamine blue were to be considered as an activator of the mesenchyme, as it has been assumed on the basis of histologic findings indicating a proliferation of fibroblasts following isamine blue injections.[948]

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[932] P. Hüssy: Zentralbl. f. Gynäk. *42*: 479 (1918).

[933] S. Russ & G. M. Scott: Proc. Roy. Soc. London, s. B *118*: 316 (1935).

[934] R. Roosen: Ztschr. f. Krebsforsch. *21*: 348 (1924); *22*: 480 (1925); *24*: 33 (1927); Prakt. Karzinomblätter *1*: 17 (1933); Deutsche med. Wchnschr. *58*: 229 (1932).

[935] H. Bernhardt & G. B. Strauch: Ztschr. f. Krebsforsch. *26*: 361 (1924).

[936] H. Bernhardt: Ztschr. f. Krebsforsch. *27*: 221 (1928); Klin. Wchnschr. *7*: 756 (1928).

[937] G. A. Kreuzwendedich von dem Borne & R. E. J. Ten Seldam: Ztschr. f. Krebsforsch. *36*: 97 (1932); Nederl tijdschr. v. geneesk. *2*: 1226 (1932).

[938] Y. Kimura: Tr. Jap. Path. Soc. *18*: 589 (1929).

[939] S. Kisimoto: Jap. J. Obst. & Gynec. *23*: 72 (1940).

[940] T. Minegishi: Tr. Jap. Path. Soc. *25*: 719 (1935).

Methylene blue has been the subject of several therapeutic attempts, mainly because of its ability to act as oxygen carrier, of which action a favorable influence on the tumor metabolism was anticipated. As long ago as 1906 it was reported that administration of this dye caused growth cessation of inoperable internal tumors, sometimes prolonging the life of

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[941] E. Boyland & M. E. Boyland: Biochem. J. *33*: 618 (1939).
[942] R. Roosen: Ztschr. f. Krebsforsch. *26*: 461 (1928).
[943] G. A. Kreuzwendedich von dem Borne: Nederl. tijdschr. v. geneesk. : 3267 (1931).
[944] H. Baltzer: Deutsche med. Wchnschr. *54*: 2054 (1928).
[945] C. L. Karrenberg: Klin. Wchnschr. *7*: 1269 (1928).
[946] H. Hamperl: Ztschr. f. Krebsforsch. *49*: 348 (1939).
[947] R. Roosen: Ztschr. f. Krebsforsch. *27*: 359 (1928); *32*: 526 1932).
[948] H. Cramer & I. G. Wallbach: Klin. Wchnschr. *9*: 41 (1930).

the patients, but never resulting in complete cure.[949] More definite therapeutic benefits have been claimed to follow a methylene blue treatment of skin cancer, although the healing process required a longer time than in cases treated with roentgen rays,[950] and therefore this safer procedure will certainly not be replaced by the dye therapy. A clinical observation has been reported, referring to a single case history, viz., of a patient suffering from gastric cancer who benefited to an unexpected degree from a treatment, continued for twenty-two months and consisting of peroral administration of methylene blue and ferrum reductum combined with subcutaneous oxygen injections.[951] Very recently, methylene blue has been employed as a supporting measure in the treatment of cancers of the oral cavity by means of nicotinic acid, but it was stressed that the beneficial results observed were principally induced by the nicotinic acid.[952] As to animal experimentation, no definite therapeutic value of methylene blue has been established. The intratumoral injection alone, a very unspecific form of application, was found to cause regression of rat tumors,[953] but only slightly retarded the growth of mouse and rabbit tumors,[954, 989] or was completely ineffective,[862] whereas intravenous methylene blue injections did not affect the growth of rabbit sarcoma, although it increased the radiosensitivity of the tumor.[989] According to a recent report, methylene blue inhibited the hepatoma formation in rats receiving o-aminoazotoluene; this effect was especially marked when the dye was fed simultaneously with the carcinogen.[955]

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[949] A. Jacobi: J. A. M. A. *47*: 1545 (1906).

[950] A. H. Roffo: Ztschr. f. Krebsforsch. *37*: 1 (1932); Bol. inst. de med. exper. para el estud. y trat. d. cáncer *7*: 374 (1932).

[951] J. Cholewa: Acta Cancrol. *1*: 243 (1935).

[952] R. Willheim & F. Bocobo: Acta med. Philipp. *2*: 445 (1941).

[953] M. M. Brooks: Proc. Soc. Exper. Biol. & Med. *30*: 1001 (1933).

[954] H. Nakamura: Tr. Soc. path. jap. *25*: 721 (1935).

[955] S. Ito: Gann *35*: 182 (1941).

Some azo dyes have been tested on account of their ability to influence metabolic processes and their lack of toxicity, such as ethyl red, isoquinoline red and Magdala red. But while these substances regularly inhibited the tumor glycolysis in vitro, the results of animal experiments were too inconsistent to encourage their practical use.[956, 957] On the other hand, a definite affinity to neoplasms, which has been ascribed to naphthalene sulfonic acids,[958] does not seem to lend itself to therapeutic experiments, because of the high toxicity of these dyes which strongly inhibit the blood coagulation.

The use of fluorescent dyes for sensitizing the tissues and causing destructive effects, when the latter are exposed to suitable radiation, has been tried in skin cancers with good results, by applying eosin to the tumors and subjecting them to ultraviolet light.[959] More recently, beneficial effects were reported in skin cancers, as a result of fluorescein application combined with roentgen radiation.[960, 961] In agreement with this, a growth inhibition of rabbit and rat tumors was observed when eosin was applied externally to tumors or by subcutaneous injections, when this was followed by exposure to ultraviolet light.[962]

Organic arsenic compounds. Into this group belong the first synthetic chemotherapeutic compounds, directed specifically against spirochetoses, which were discovered by Ehrlich and his collaborators. As is frequently apparent in the history of medicine, the success of a new therapy in one field is frequently followed by an almost indiscriminate transfer of the

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[956] L. Karczag: Biochem. Ztschr. *230*: 411 (1930).

[957] L. Karczag & C. Sellei: Biochem. Ztschr. *230*: 420 (1930).

[958] K. Kottmann: Schweiz. med. Wchnschr. *65*: 533 (1935).

[959] A. Jesionek & H. von Tappeiner: Deutsches Arch. f. klin. Med. *82*: 223 (1905).

[960] S. M. Copeman, F. Coke & C. Gouldesbrough: Brit. M. J. *2*: 233 (1929).

[961] S. M. Copeman: Internat. Conf. Cancer, London, p. 256 (1928).

[962] H. Nakamura & S. Niwa: Tr. Jap. Path. Soc. *23*: 712 (1933).

treatment to many other pathologies, without sufficient reason. In such a manner, organic arsenic compounds have been tried in the therapy of human malignancy; some of these attempts did not yield favorable results,[963] but another author claimed to have accomplished considerable improvements by injections of atoxyl in sarcoma patients, and also in some cases of skin cancer, frequently in combination with roentgen therapy; internal tumors mostly proved refractory.[964] Similar experiences have been reported from time to time, but extended to a few clinical cases only, and are therefore insufficient to establish a definite value for this treatment. Neither have uniform and conclusive results been derived from animal experiments in which various arseno-benzene compounds (arsphenamine, neosalvarsan, neoiacol) were tried on different tumors. The takes of Ehrlich carcinoma were found to be suppressed in forty per cent of mice which had received injections of these substances previous to the inoculation,[965] and a similar treatment, administered after the transplantation of various animal tumors, inhibited the neoplastic growth, particularly of Kato rabbit sarcoma.[966-968] Regressions of Flexner-Jobling rat carcinoma and Bashford mouse carcinoma were observed in forty-five to fifty-three per cent of animals treated with salvarsan, if the injections were started soon after the tumor inoculation.[969] Another worker, however, found injections of an arsenobenzene compound rather as growth promoting in Ehrlich mouse car-

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963. H. Hollaender & D. Pecsi: Wien. med. Wchnschr. *59*: 210 (1909).

964. F. Blumenthal: Strahlentherapie *3*: 523 (1913).

965. G. Montemartini: Tumori *14*: 1 (1928).

966. A. Tanaka: Tr. Soc. path. jap. *23*: 692 (1933); *25*: 711 (1935).

967. A. Tanaka, S. Yamamoto & I. Oouchi: Mitt. a. d. med. Akad. zu Kioto *11*: 1221 (1934).

968. A. Tanaka, I. Oouchi, S. Yamamoto & K. Tukada: Tr. Soc. path. jap. *25*: 693 (1935).

969. S. Yamamoto: Mitt. a. d. med. Akad. zu Kioto *26*: 459 (1939).

cinoma.[970] No effect from this treatment was observed, as far as the development of benzpyrene tumors in mice was concerned.[971] A slight growth stimulation has been observed in spontaneous mammary carcinoma of mice following salvarsan administration, and consequently caution has been urged regarding the use of this drug in luetic cancer patients.[972] These conflicting results, obtained with organic arsenic compounds, are reminiscent of the ambivalent action of inorganic arsenic compounds, which, as discussed in the preceding chapter, may exert both a tumor promoting and a tumor inhibiting effect, but in any case, this does not encourage the use of such preparations in cancer therapy.

Another synthetic drug of complex organic structure, viz., germanine, used as a specific remedy for sleeping sickness, has been claimed to cause some improvement in hopeless cancer cases.[973]

Alkaloids. Quinine, which has proved a specific and reliable chemotherapeutic agent in the fight against malaria, has been introduced into cancer therapy for various reasons. On the one hand, it was assumed that an allegedly less frequent cancer incidence in regions where malaria is endemic might be connected with the generous use of this alkaloid as a remedy, and as a prophylactic measure, whereas other authors obviously were guided by the belief that parasites, belonging to the group of protozoa, were responsible etiologically for neoplasia, and therefore that quinine would represent an adequate medication. Without digressing into any theoretical discussion of the more than questionable validity of these premises, it is stated that clinical experiences did not furnish convincing proof for a usefulness of quinine therapy in malignant tumors. Although some beneficial effects, such as alleviation of pain, lessening of secretion, and even involution of tumors, were claimed occasionally to result from quinine administration,[974] most of the authors arrived at entirely negative results.[927] As far as laboratory experiments were undertaken, a growth inhibition of neoplastic cells was observed in tissue cultures when quinine salts had been added,[975] and in a recent study twenty-three quinine derivatives have been tested in a similar way. It was found that the growth inhibition increased with the number of carbon atoms in the side chain at the 6 position of the quinoline ring, eucupin and vuzin being the most effective.[976] It must, however, be taken into account, that quinine and its derivatives represent drastic protoplasma poisons, suppressing most of the enzymatic processes, and therefore it is doubtful whether the findings mentioned could be reproduced in vivo, that is, if the required large amounts of alkaloid could be brought to act upon tumor cells without endangering the rest of the organism. In fact, most of the successful animal experiments used a technique by which tumor cells were directly exposed to the drug, either by injecting a one per cent quinine sulfate solution into mouse tumors with resulting regression of the neoplasms,[977] or by incubating Ehrlich mouse carcinoma for six hours with a three per cent quinine solution, after which procedure the inoculation failed to produce tumors.[978] The authors of the latter communication also claimed to have arrested the growth of, or to have cured, mouse carcinomas by the daily injections of 0.5 mg. of quinine,[979] but other studies failed to demonstrate a favorable effect of quinine administration in tumor-bearing animals.[980, 981] Direct contact of the quinine derivatives quinidine and hydrocupreines with tumor material decreased the yield of subsequent inoculation, and these compounds were found to cause carcinolysis in vitro.[840]

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[970] L. Bertellotti: Minerva med. *2*: 602 (1935).

[971] L. Bertellotti: Arch. ital. med. sperim. *4*: 97 (1939).

[972] W. C. Hueper & S. Itamo: Am. J. Cancer *17*: 106 (1933).

[973] H. Auler: 2nd Internat. Cancer Congress, Brussels *1*: 361 (1936).

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[974] Jaboulay: abstr. Berl. klin. Wchnschr. *38*: 592 (1901).

[975] H. Vollmar: Arb. Inst. Exper. Ther. Frankfurt *25*: 134 (1931).

[976] N. Sofue: Folia Pharmacol. Jap. *31*: 1 (1941).

[977] H. Hirschfeld: Ztschr. f. Krebsforsch. *16*: 33 (1919).

[978] A. Levi & E. Bellodi: Boll. soc. Ital. Biol. Sper. *4*: 1175 (1929).

[979] A. Levi & E. Bellodi: Arch. di. farmacol. sper. *50*: 160 (1930).

Of other alkaloids, pilocarpine has been reported to cause regression of, or to inhibit, mouse carcinomas and rat sarcomas, which phenomena have been connected with the vagotropism of this substance,[982] although in another study the formation of tar cancer in rabbits was found to be impeded when the vagus-inhibitory alkaloid atropine, or the sympaticotropic substances ephetonine and ephedraline were administered.[983] Pending a more thorough experimental analysis of these findings, as well as of a tumor inhibiting action claimed as being exerted by ergotoxin,[984] it can only be suggested in a general way, that the alkaloids named caused a severe disturbance of the whole organism, affecting principally the nervous and vascular supply of vital organs, and thus indirectly interfering with the growth rate of the neoplasms.

Ample literature has been contributed to the problem of colchicine in relation to neoplasia. The investigations reported have been prompted, on the one hand, by an incidental observation that cancer patients, who suffered from gout and received colchicine as an adequate remedy, seemed to benefit from this medication also regarding their neoplastic condition.[985] On the other hand, very interesting results were obtained in extensive histologic investigations, performed by Dustin and his collaborators,[986] who studied the effect of

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980. S. Fränkel & E. Fürer: Wien. klin. Wchnschr. *29*: 198 (1916).
981. F. Ottensooser: Ztschr. f. Krebsforsch. *35*: 331 (1932).
982. H. U. Hirsch-Hoffmann: Deutsche med. Wchnschr. *54*:. 309 (1928).
983. A. Tavares: Compt. rend. Soc. d biol. *118*: 1507 (1935).
984. V, Ghiron: Boll. acad. med., Roma *59*: 367 (1933).
985. E. C. Amoroso: Nature, London *135*: 266 (1935).
986. A, P. Dustin: Arch. f. exper. Zellforsch. *22*; 395 (1939),

colchicine on the mitotic process in animal and plant cells. The normal course of mitoses appeared to be changed in some cells in a characteristic way, interpreted by the authors as an arrestation in the metaphase, thus preventing the continuation of the cell division. The experimental work, however, did not lead to the results hoped for, which would have proved a usefulness of colchicine for tumor therapy. Although the mentioned influence of the alkaloid on mitoses of rat liver cells has been confirmed,[987] this effect was found more marked in this normal tissue than in transplanted rat sarcomas,[988, 989] for which the poor vascularization of the tumor was thought to be responsible.[989] Spontaneous tumors and tar cancers of mice failed to respond to colchicine treatment,[990] and in studies in which regressions of Shope papillomas of the rabbit, an infectious, non-malignant tumor, were observed following colchicine administration, very high doses of the drug had to be employed for this result.[991, 992] Toxic colchicine doses caused hemorrhages in transplanted mouse and rat tumors, accompanied by a reduced vitamin C content, and a decreased glycolytic activity of the tumors,[993] whereas spontaneous tumors failed to react to this treatment.[846] In spite of an observed inhibition of mitoses, effected by toxic colchicine amounts, the Crocker mouse sarcomas resumed the original growth rate when the administration of the drug was stopped,[994] and colchicine quantities, which were seen to in-

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987. A. M. Brues & A. Cohen: Biochem. J. *30*: 1363 (1936).

988. A. M. Brues & E. B. Jackson: Am. J. Cancer *30*: 504 (1937).

989. P. A. Clearkin: J. Path. & Bact. *44*: 469 (1937).

990. K. T. Poulson: Norsk. Mag. Laegevidensk *96*: 735 (1935); abstr. Am. J. Cancer *30*: 155 (1937).

991. A. Peyron, B. Lafay & Kobozieff: Bull. Assoc. franç. p. l'étude du cancer *25*: 874 (1936).

992. A. Peyron, G. Poumeau-Delille & B. Lafay: Bull. Assoc. franç. p. l'étude du cancer *26*: 624 (1937).

993. E. Boyland & M. E. Boyland: Biochem. J. *31*: 454 (1937).

994. R. J. Ludford: Arch. f. exper. Zellforsch. *18*: 411 (1936).

hibit the growth of tissue cultures of the mouse sarcoma 37, did not affect the tumor growth in vivo.[989] In Brown-Pearce rabbit carcinomas, colchicine administration caused an increase of metaphases, but since this effect was subject to unpredictable variations, its therapeutic utilization was not deemed promising.[994a] Better results have been reported from the colchicine treatment of malignant lymphoid tumors of mice, namely regressions of the neoplasms and prolongation of life in about fifty per cent of the animals treated.[995] Other investigators confirmed the fact that tumor growth could be retarded only by almost lethal colchicine doses;[996, 997] following this treatment hemorrhages have been observed in spontaneous and transplanted mouse tumors which resembled those caused by injection of bacterial filtrates (Shwartzman phenomenon).[996] Tissue cultures of mouse breast carcinoma, though inhibited in growth by low colchicine concentrations, had not lost their vitality, as was demonstrated by their successful re-implantation to mice,[998] and it was stressed that only high colchicine doses were effective in vivo, causing necroses of the tumors, but never complete regression.[999] An experimental procedure, differing from the studies enumerated, has been employed in an investigation on the Flexner-Jobling rat carcinoma. If animals received subcutaneous colchicine injections, and subsequently distilled water was injected directly into the tumors, they receded in a great percentage of the treated animals, whereas intratumoral water injection, without colchicine, failed to have the same effect.[1000] Because of the previously mentioned alterations of the mitotic course, brought about by colchicine, it has been assumed that the anomalous cell forms occurring might possess a greater radiosensitivity,[1001] but suitable experiments yielded far from uniform results. While some authors stated that colchicine enhanced the radiosensitivity of rat carcinoma in vitro and in vivo,[1002] other studies indicated only a slight increase of the radiation effect due to administration of colchicine,[999] or completely failed to demonstrate such a phenomenon.[997] As far as human pathology is concerned, it has been reported that in a patient suffering from gastric cancer, skin metastases, in the surrounding of which 0.1 mg. colchicine had been injected, completely disappeared after four roentgen irradiations, whereas other skin metastases of this patient, not treated with colchicine, did not respond to radiation in such a favorable manner.[1003] Another communication, however, extended to a discussion of four advanced cancer cases treated with toxic doses of colchicine, two of which died of colchicine poisoning, while in the other two the treatment was continued until death occurred due to the disease. The post mortem examination revealed extensive necrosis and sloughing of the tumors, but histologic analysis showed a stimulated growth of the tumor periphery.[1004] Reviewing these results, the practical usefulness of colchicine seems highly doubtful in view of the toxic doses necessary for achieving growth cessation of the tumors, and in view of the temporary nature of this effect. It remains to be seen whether substances of related

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[994a] B. DuBilier & S. L. Warren: Cancer Research *1*: 966 (1941).

[995] F. J. Lits, A. Kirschbaum & L. C. Strong: Proc. Soc. Exper. Biol. & Med. *38*: 555 (1938); Am. J. Cancer *34*: 196 (1938).

[996] H. B. Andervont: J. Nat. Cancer Inst. *1*: 361 (1940).

[997] A. M. Brues, B. B. Marble & E. B. Jackson: Am. J. Cancer *38*: 159 (1940).

[998] R. Tennant & A. A. Liebow: Yale J. Biol. & Med. *13*: 39 (1940).

[999] J. W. Hirshfeld, R. Tennant & A. W. Oughterson: Yale J. Biol. & Med. *13*: 51 (1940).

1000. M. F. Guyer & P. E. Claus: Proc. Soc. Exper. Biol. & Med. *43*: 272 (1940).

1001. F. J. Lits: Arch. internat. méd. exper. *11*: 811 (1936); abstr. Am. J. Cancer *31*: 30 (1937).

1002. M. F. Guyer & P. E. Claus: Proc. Soc. Exper. Biol. & Med. *42*: 565 (1939).

1003. F. Th. v. Brücke & E. F. v. Hueber: Klin. Wchnschr. *18*: 1160 (1939).

1004. L. Seed, D. P. Slaughter & L. R. Limarzi: Surgery *7*: 696 (1940).

chemical nature may be found, exhibiting a lesser toxicity and a greater effectiveness as to growth inhibition and, possibly, as to stimulation of the radiation effect. In this connection, seventy-five phenanthrene derivatives, structurally resembling colchicine, have been tested in transplanted and spontaneous mouse tumors, with the result that injections of the compounds tested caused regression of the transplanted tumors in up to fifty per cent of the animal series, but had no effect on the spontaneous tumors.[1005]

An organic compound of paramount importance for plant biology, *chlorophyll,* has been tried within the last years in cancer patients. Some beneficial effects of general improvement have been reported [1006-1008] and the possibility of supporting radiation therapy by chlorophyll (possibly involving a photo-catalytic effect) has been considered.[1009] However, the general pharmacology of chlorophyll and experimental work dealing with its effect on neoplasms have not advanced far enough for a planned undertaking of clinical treatment and its correct interpretation.

The survey, given here, has not included all those measures which are usually taken, or have been recommended, in the clinical treatment of neoplasms, for the alleviation of pain and other secondary conditions as they may arise during these diseases, since these therapeutic activities are not affecting, or expected to affect, the cancerous process itself. A presentation, reviewing the symptomatic treatment of cancer, has recently been given,[1010] and it may be mentioned here that, on the basis of experimental studies in which several new compounds, effective for relief of pain in inoperable cancer patients were prepared and tested, a methyldehydromorphine has been found to present promising aspects for such purposes.[1011]

[1005] F. C. Turner: Pub. Health Rep. *54*: 1279 (1939).

[1006] A. Plagniol: J. de méd., Paris *53*: 664 (1933).

[1007] R. Dupont & G. Duhamel: Bull. Assoc. franç. p. l'étude du cancer *24*: 15 (1935).

[1008] M. Delarue: Bull. Assoc. franç. p. l'étude du cancer *24*: 27 (1935).

[1009] I. David-Galatz: Rev. de laryng. 54: 1027 (1933); abstr. Am. J. Cancer *24*: 133 (1934); Progrès méd. p. 583 (1935); abstr. Am. J. Cancer *30*: 786 (1937).

[1010] N. Treves: The Med. Clin. of North America, Vol. *24*: 595 (1940).

Summary

Some doubts may arise in the mind of the reader as to whether one is justified in uniting under one heading such different subjects as the organic composition of malignant tumors, carcinogenic substances, and attempts at organic chemotherapy of neoplasia—apparently because of the extraneous fact that the topics dealt with have a relation to organic chemistry in common. Even if it is not denied that such a conventional and schematic viewpoint has partly influenced the arrangement of the material surveyed, there are, to the authors' belief, deeper and more intrinsic reasons involved which not only permitted, but even necessitated, the conjunction of these fields of cancer research. This view is based not so much on the present state of knowledge of the cancer problem, valuable contributions to which have been listed in this chapter, but more so on future trends of experimental work which may be expected, and which can be predicted with a reasonable degree of certainty.

While enlarging on this concept, it is deemed appropriate to review, in brief, the principal conclusions which may be derived from the studies discussed.

Turning to the organic chemistry of neoplasia, a clear distinction has been made between those deviations from the norm, apparent in malignant tumors and in the diseased body, which are merely secondary phenomena of tissue decomposi-

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1011. L. E. Lee, Jr.: J. A. M. A. *116*: 218 (1941).

tion and general cachexia, and those alterations which seem more closely connected with the neoplastic process. It must, however, be understood that none of the latter findings, referring to carbohydrates, proteins or lipids, can be considered as characteristic for malignant tumors, in the sense that similar qualitative or quantitative deviations have not also been encountered in non-malignant conditions. The anomalous findings of organic tumor chemistry have rather been found to be subordinate to one specific quality of neoplasms, namely their excessive and autonomous growth rate, and their correct and productive interpretation is obviously hampered by our insufficient knowledge regarding the factors governing the normal growth pattern. At this point, valuable information may be hoped for from thorough investigations of nuclear chemistry, a field which appears to have escaped the exact elaboration which it seems to deserve by virtue of the close relationship between cellular division and growth processes.

At first sight, the work dealing with organic carcinogens has led to much more concrete achievements. The brilliant work done is by no means to be belittled as to its significance, since only two decades ago it would have been almost unbelievable that organic substances of defined chemical structure, synthesized in quantity in the laboratory, would induce the development of malignant tumors, and open to observation the sequences of this mysterious process. But it must be emphasized again that these accomplishments cannot possibly represent an end in itself, but must be regarded as a challenge toward the solution of new and fundamental problems. Paramount among these, is the question whether and to what degree substances, similar to the synthetic carcinogens, are participating in, and are responsible for, the etiology of neoplasia in general. And at this point of the problem, the support of organic-analytical studies appears indispensable, particularly regarding those body substances, such as sterols, some hormones, and vitamins, which exhibit a close chemical relationship to the carcinogens. At the same time, it will be necessary to bear in mind that the carcinogenic mechanism apparently requires an active participation of the whole organism, or, in other words, that the transformation of a normal cell into a malignant one is not solely decided by the local reaction to a certain carcinogenic stimulus, but is, also, and perhaps primarily, determined by the response of the rest of the body, in the sense that defense mechanisms, striving to uphold the normal regulatory influence on all cells against disturbing factors, are able to prevent the development of neoplasms, or fail to do so. Some insight into these correlations has already been gained from studies detailed above, and continued investigation of the metabolism of the carcinogens might yield further elucidation of the question.

It is easy to comprehend that these endeavors, if successful, will bear directly on the problem of effective chemotherapy of malignant tumors, insofar as a basis would be furnished for strengthening the forces of the body, counteracting neoplasia, both in the form of prophylactic measures and as a supporting therapy, supplementing local treatment by surgery and radiation, which in themselves unfortunately do not protect against recurrence or a new spread of the disease. As has been noted, none of the chemotherapeutic experiments, to date, could be credited with such desirable accomplishments, the reason for which may be seen in their insufficient theoretical foundation, necessitating a rather haphazard manner of selecting the compounds tried. But it may be surmised with some optimism that, once a clearer insight into the interaction of carcinogens and cell constituents has been acquired, a more reasonable choice will be effected among the almost unlimited resources of organic chemistry for selecting compounds which might prove helpful in cancer therapy.

Thus, it is thought, the progress of cancer research, attacking the problem from different angles of organic chemistry—analytic determinations, carcinogenic compounds, chemotherapeutic substances—might, in the not too distant future, lead to the establishment of a much closer relationship between these branches of investigations than it has been possible to outline on the basis of the work accomplished thus far.

CHAPTER III

PHYSICAL CHEMISTRY

SINCE the beginning of this century, a concept that met with the ever increasing support of scientists recognizes that the most intricate processes of cellular biology can be comprehended to much higher degree if, apart from attempts at determining the inorganic and organic composition of a biologic substrate, its physico-chemical status is taken into consideration. Applications to biologic problems of this comparatively recent branch of chemistry have led to an advanced understanding of physiologic processes, e.g., of glandular secretion, intestinal resorption, formation of lymph and spinal fluid, excretion of urine, and of pathologic conditions, such as edema, transudation and exudation, inflammation, formation of calculi, etc. An advantage common to most physico-chemical investigations may be seen in the fact that the methods and measurements employed can be applied to the intact biological material. Hence the results obtained may be regarded as much more illustrative of the conditions prevailing in the living cell than the data which are derived from studies necessitating the previous destruction of the cellular entity. Under these circumstances, it seemed logical to include tumor pathology among the problems to the clarification of which physical chemistry might represent a suitable approach. Most of the pertinent contributions are of recent date and have, as yet, by no means covered the whole field of physical chemistry in a systematic manner; therefore the following sections of this chapter, into which the recorded material has been divided, must be regarded as a few bricks only, from which, at a future date, the complete edifice may be erected.

1. *Cell Permeability, Cytolysis and Hemolysis.*

The phenomenon of a rapid growth rate, as exhibited by neoplasms, of necessity implies an abundant intake of nutritive material by the tumor cells, and therefore it suggested, a priori, the possibility of an increased cellular permeability as one of the factors facilitating the entry of needed supplies from the cell environment. Actually, several investigations, directed at this problem, indicated an increase of the permeability of cancer cells.[1, 2] It is notable that in one of the older studies this anomaly was found to extend to the metastasis-free liver of animals also, shortly after tumors had been inoculated.[2] The conclusion that an augmented permeability of neoplastic tissue exists has also been inferred in an indirect way, i.e., from measurements of the electrical polarization and of the electrical resistance of the tumors. The calculated ratio of polarization/resistance has been found markedly reduced in tumors, as compared with normal tissue, and the explanation of this finding was seen in the easier passage through the tumor cell membranes of those ions which are responsible for the electrical polarization.[3] A more direct approach has been chosen in other studies by employing the diffusion of phosphates, determined in tumor slices, as indicator of their permeability; in these experiments, during a prolonged period of observation, a gradual diminution

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[1] M. Wolf: Compt. rend. Acad. d. sc. *176*: 1932 (1923).

[2] P. Mendeleéff: Compt. rend. Soc. de biol. *94*: 1277 (1926).

[3] N. Waterman: Ztschr. f. Krebsforsch. *19*: 101 (1923); *27*: 228 (1928).

of the phosphate diffusion was found, and the diffusion rate could be restored by the addition of potassium cyanide or by the addition of large amounts of anesthetics (phenyl urethane). However, since under these conditions cell death was observed morphologically, the secondary increase of the tumor permeability was interpreted as a lethal reaction.[4] Using the same method, the influence of roentgen radiation was investigated and it was reported that, in contrast to normal tissue, this interference was followed by a reduction of the permeability of neoplastic tissue,[5, 6] a fact which might partly explain the deleterious effect of radiation on tumors. The opinion has been voiced that the increased permeability of tumor cells might be an expression of an excitation, in a manner similar to the form in which it occurs under physiological conditions, e.g., in the muscle, although in the normal organs this excitation has a reversible character, depending on the metabolic phase existing.[7] Hence the permanently increased permeability of neoplastic cells might be connected with their metabolism which is of a predominantly anoxybiontic nature; this view seems supported by the observation of an increased permeability of normal tissues (liver and kidney), induced by .poisoning with potassium cyanide.[7] The assumption of an augmented and changed permeability of tissue has also been derived from studies dealing with the so-called mitogenetic radiation, a phenomenon discussed in other parts of this book; in connection with permeability, it was claimed that the enzymes, or other factors, responsible for this radiation, diffuse from tumors into the surrounding medium, whereas such an effect does not take place with normal tissue.[8] But since the entire phenomenon of mitogenetic radiation is still highly controversial, the value of the mentioned findings appears to be inferior to that of the results based on other methods. Finally, a negative finding must be recorded, inasmuch as in tissue cultures the permeability of normal and of sarcomatous fibroblasts for water has not been found to differ,[9] a fact which might imply that the permeability of tumor cells may be increased for some substances, while not for others, but at the same time this statement makes a re-examination of the earlier findings highly desirable. Negative results were also obtained in experiments testing the effect of choleic acid-compounds of carcinogenic hydrocarbons on cell permeability; sea-urchin eggs, or red blood cells of mice or cattle, showed no change of their permeability after exposure to these compounds.[10]

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[4] H. Lange & N. Henning: Arch. f. exper. Path. u. Pharmakol. *131*: 70; 75; 115; 199 (1928).

[5] M. A. Magath: Ztschr. f. Krebsforsch. *25*: 122 (1927); Vopr. Onkol. *3*: 9 (1930); abstr. Ztschr. f. Krebsforsch. *34*: Ref. 19 (1931).

[6] M. A. Magath & M. J. Kolomijetz: Ztschr. f. Krebsforsch. *30*: 457 (1930).

[7] E. Gellhorn: Das Permeabilitätsproblem. Berlin, Springer 1929.

A changed membrane permeability of the erythrocytes of cancer patients has been indicated by the observation of a diminished potassium diffusion into the blood serum from red blood cells derived from patients afflicted with neoplasia, but also from those of patients suffering from other diseases (icterus, endocrine disturbances) or of pregnant women.[11] Another investigator claimed that in cancer blood the normal migration of potassium from erythrocytes into the plasma was even reversed, by means of a re-adsorption of potassium to the red blood cells,[12] but only more experiences will reveal whether this behavior is characteristic for cancer patients.

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[8] A. Gurwitsch: Die mitogenetische Strahlung, Vienna, Springer 1932.

[9] A. M. Brues & C. McTiernan Masters: Am. J. Cancer *28*: 324 (1936).

[10] B. Lucké, A. K. Purpart & R. A. Ricca: Cancer Research *1*: 709 (1941).

[11] P. Spiro: Verhandl. d. Gesellsch. f. inn. Med., p. 355 (1930).

[12] L. Koster: Acta med. Scandinav. *93*: 420 (1937).

In dealing with the permeability of cancer cells, it will be recollected from the first chapters that both inorganic and organic deviations of the tumor composition have been correlated with this quality. As to inorganic constituents, the potassium calcium ratio has been regarded as a decisive factor, insofar as calcium has the ability to tighten cell membranes, whereas potassium promotes hydration and permeability. Accordingly, the addition of calcium to tumor tissue has been found to cause a reversion of the tumor cell permeability to more normal relations, as measured by the electrical polarization.[8] In regard to organic constituents, it is recalled that the great significance of lipids in the structure of cell membranes, and consequently in the cell permeability, has been emphasized in the preceding chapter, and especial attention has been called to the cholesterol increase in neoplasms. In addition, the augmented phospholipid content of tumors was made responsible for their increased permeability, and these relations were assumed to be connected with their rapid growth.[13]

A broader theory regarding the factors which determine changes of permeability and connect them with the cancer problem has been developed on the basis of work with tissue cultures.[14, 15] These studies revealed that, in mixed cultures of epithelial and of connective tissue, the growth of the first named elements was enhanced by the following measures: (1) by addition of surface-active substances, particularly of cholesterol, (2) by hypotonic solutions, (3) by the presence of hydration-promoting ions, which increased in effectiveness in accordance with Hofmeister's series. All these interferences were claimed as being followed by an increased permeability of the cell membranes. A possible close connection of these findings with the cancer problem seems to be given by the facts that, on the one hand, the measures mentioned resulted in an epithelial growth displacing the connective tissue elements, and that, on the other hand, some of the experimentally induced conditions, influencing the cell permeability, appear to be present in malignant tumors, such as an accumulation of cholesterol, an augmentation of surface-active substances, and the prevalence of the hydration-promoting potassium over the dehydrating calcium. Similarly, the opinion was expressed at an earlier date that a possible connection between the lipid solubility of carcinogenic agents and between resulting changes of the cell permeability should be borne in mind. "The lipid cell walls and the cell body undergo changes of physico-chemical nature by the action of the (carcinogenic) stimulus, and a disturbance of the osmotic balance between cell body and the surrounding humoral medium results. The consequence of this physico-chemical alteration is a grave change-over of the cell mechanism, eventually transforming the biologic cell character in such a way that the blastoma cell, exhibiting autonomous growth, results." [16] While the general validity of these theories and of other conceptions,[17] assigning to the phenomenon of cell permeability a central position within the cancer problem, are impaired by their predominantly speculative nature, insufficiently supported by experimental evidence, it must be conceded that, at least as far as the synthetic carcinogens are concerned, most of them have been found to be lipid soluble, and hence may affect the cell membranes.

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[13] W. B. Bell: Lancet *2*: 1003 (1925).

[14] J. Traube & E. Knake: Ztschr. f. Krebsforsch. *42*: 324 (1935).

[15] E. Knake: Ztschr. f. Krebsforsch. *42*: 329 (1935).

Attempts have been made to produce changes of the general permeability in tumor-bearing animals by means of

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16. L. Wacker; quot. F. Klinge: Ergebn. d. inn. Med. u. Kinderh. *29*: 152 (1926).

17. W. Kopaczewski: Permeabilité cellulaire et problème du cancer, Le Francois, Paris 1934.

interferences disturbing the fluid exchange in the body. Massive bleeding of the animals, parenteral administration of proteins and other lyophilic colloids, or histamine injections were employed for this purpose, but no definite effects of these measures were noted as to the tumor growth.[18] It must, however, be taken into consideration that, in all experiments aimed at specific alterations of physico-chemical qualities, the strongly opposing tendency of the organism to maintain the status quo represents an essential difficulty, and that no proof has been furnished as to whether the desired change—in this case a reduction of the cell permeability—had been actually achieved.

It has been demonstrated that tissue extracts may exert an effect on phenomena connected with cell permeability, inasmuch as their intracutaneous injection enhanced the diffusion of subsequently injected diphtheria toxin or India ink, thus spreading those substances over larger areas than in untreated skin; in this respect, testis extract was most active, but a marked "spreading effect" was also observed following the administration of tumor extracts, the activity of which seemed to run parallel to their growth rate.[19] Hence it may be assumed that neoplastic tissue—and certain normal organs to an even greater degree—contain some factors which promote the spread, viz., the diffusion of colloids in the tissue—a phenomenon, the significance of which it may be possible to evaluate in connection with further investigations.

The permeability of cell membranes is known to be of decisive importance for the phenomena of *cytolysis* and *hemolysis,* respectively. However, at this point only those lytic effects will be gone into, which depend on physico-chemical factors, whereas those of immunological nature will be dealt with in a later chapter. The specific relations involved in the cytolysis of cancer cells by the action of blood serum are discussed in the last chapter under the heading of "carcinolysis"; here we need only refer to the claim that succinic acid and homologous saturated dicarboxylic acids are endowed with a specific carcinolytic property,[20] a finding which seemed substantiated by studies with neoplastic tissue cultures.[21] On the other hand, it was reported that cancer cells prove resistant against solutions of potassium cyanide, formamide and saccharine, although these substances caused cytolysis of normal cells.[22]

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[18] M. J. Shear: Am. J. Cancer *25*: 66 (1935).

[19] E. Boyland & D. McClean: J. Path. & Bact. *41*: 553 (1935).

The objects most frequently studied for cytolytic investigations are erythrocytes, because of their easy accessibility and on account of their comparatively constant qualities. The usual clinical examination is limited to the fragility test, i.e., the determination of the lowest hypotonic salt concentration which still fails to produce any indication of hemolysis, and of the highest concentration, respectively, causing complete hemolysis. It has been reported that red blood cells, derived from patients with advanced uterine or vaginal cancer, exhibited, in this test, an increased resistance against the hemolytic action of hypotonic solutions, and this observation was considered as indicating a defensive reaction of the organism against hemolysins, which are supposedly formed by the tumors.[23] A similar finding of a diminished susceptibility of erythrocytes to hemolysis has been noticed in intestinal neoplasms,[24] but it seems beyond any doubt that this behavior is certainly not primarily connected with neoplasia,[25] since all secondary anemias of marked degree result in an analogous alteration of the osmotic resistance of the red blood cells. Indeed, it was established in experiments with tumor-bearing rats that the diminished hemolyzing tendency of the erythrocytes of the animals was paralleled by a decrease of the hemoglobin values.[26] Therefore, findings of a normal fragility of red blood cells of cancer patients [27] can be explained by the assumption that the selected cases were not, or were to a lesser degree, suffering from anemia. More characteristic alterations of the red cell fragility were claimed to become apparent when, instead of the saline solutions, a glucose-free tyrode solution was employed for the test; in this case, the first indication of hemolysis of red blood cells, derived from cancer patients, was seen in an 0.7 per cent solution, which does not affect normal erythrocytes, but complete hemolysis of the cancer erythrocytes was not induced even by an 0.2 per cent solution.[28] But more numerous investigations of this kind are required before the specific nature can be acknowledged, and the same applies to a number of results which have been reported in regard to hemolysis induced by means of various chemical substances, effects which must be clearly distinguished from hemolysis due to hypotonic solutions. It was stated that the red blood cells of animals or humans, affected with neoplasia, were more susceptible to the hemolytic action of lactic acid, or of oleic acid, respectively, than the erythrocytes of tumor-free individuals,[29] and a study, employing a more complex hemolytic agent, viz., cobra venom, seemed to indicate a different

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[20] E. Freund & C Kaminer: Biochemische Grundlagen der Karzinomdisposition, Vienna, Springer 1925.

[21] M. A. Goldzicher & T. Péterfi: Ztschr. f. Krebsforsch. *31*: 361 (1930).

[22] V. Laufberger: Biochem. Ztschr. *181*: 225 (1927).

[23] K. Schmiedlechner: Ztschr. f. Krebsforsch. *3*: 246 (1905).

[24] E. Cohnreich: Klin. Wchnschr. *5*: 1650; 2382 (1926).

[25] W. E. Künstler: Folia hemat. *49*: 21 (1933).

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26. A. Picco & L. Rigoletto: Cancro *5*: 140 (1934).

27. L. Mitrovitch: Sang *3*: 440 (1928); Compt. rend. Soc. de biol. *100*: 67 (1929).

28. J. A. Hoffmann: Časopis lék. česk. 1936: 49; abstr. Ztschr. f. Krebsforsch, *44*: Ref. 232 (1936).

29. J. Goldberger: Fol. serol. *7*: 941 (1911); abstr. Berlin. klin. Wchnschr. *49*: 310 (1912).

behavior of red blood cells of animals, according to the nature of the tumors transplanted. From this it was seen that erythrocytes of animals inoculated with carcinoma appeared more resistant to hemolysis from cobra venom than normal red cells, whereas those of sarcoma-bearing animals were more susceptible than normal ones; red blood cells derived from patients afflicted with carcinoma or sarcoma, respectively, exhibited a similar reactivity, but not as consistently as in the animal experiments.[30] Some photo-dynamic compounds, like fluorescein, erythrosin and chlorophyll have been tested as to their hemolytic activity, in part combined with exposure to ultra-violet light, and it was claimed that erythrocytes of tumor-afflicted humans and animals were four to five times more susceptible to this type of hemolysis than normal ones,[31] an observation, which, however, was contested by another worker.[32]

Studies concerned with the influence on hemolytic processes of the blood serum of cancer patients have been performed principally for diagnostic purposes, and therefore accounts of these investigations will find their place in the last chapter. Here it need only be remarked that those findings which seemed to point out a different action of normal and of cancerous serum can be easily comprehended as a result of the changed physico-chemical qualities of the blood serum of cancer patients; in this connection, reference is made principally to the surface tension, discussed in the next section, as a factor influencing the lipid structure of blood cells, and hence determining their lysis.

No specific significance can be attributed to the finding of an ether-soluble and coctostable hemolytic agent in the gastric juice of patients suffering from gastric cancer,[33] since this most probably was a decomposition product of tissue—possibly fatty acids—and, moreover, its specific occurrence in gastric cancer has been disclaimed.[34]

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[30] R. Kraus, O. Pötzl, E. Ranzi & H. Ehrlich: Wien. klin. Wchnschr. *22*: 1027 (1909).

[31] A. H. Roffo: Bol. inst. med. exper. para el estud. y trat. d. cáncer *7*: 338; 595 (1932); *9*: 200 (1932); Ztschr. f. Krebsforsch. *38*: 312 (1932).

[32] G. Galvano: Bol. soc. med.-chir. Catania *1*: 526 (1933); abstr. Ztschr. f. Krebsforsch. *40*: Ref. 137 (1934).

2. *Surface Tension, Surface-Active Substances and Adsorption.*

Surface tension belongs to the most extensively studied physico-chemical qualities in biology, by virtue of the well elaborated and comparatively simple methods available for its determination. The observations made gave rise to some interesting hypotheses as, for instance, a conception which envisaged an analogy between the tendency to increased drop formation in a liquid, in consequence of a lowered surface tension (increased surface activity), and between the tendency of cells to divide.[35] Such a theory, accordingly, would imply that tissues with an increased growth rate and accelerated mitotic activity, such as neoplasms, should exhibit a lowered surface tension. Whatever attitude may be taken regarding this theory, which certainly tends to over-simplification, the results obtained experimentally appear to agree with the conclusion drawn, inasmuch as several authors reported, in agreement, that they had found a lowered surface tension in extracts of malignant tumors, as compared with the values for normal organs.[36-39] It was stressed that malignant tumors also exhibited a higher surface activity when contrasted with benign tumors,[36] but when an attempt was made by some authors[40] to classify normal organs in the order of their surface activity, and also to see a direct relationship between the values determined and between the frequency of metastases in them, one must object that, according to clinical and pathological experiences, the formation of metastases is obviously governed by factors of an entirely different nature, that is, by the anatomical relations of blood and lymph vessels, on the one side, and by the type of the neoplasm on the other. This is shown, e.g., by the tendency to metastases occurrence in bones of patients suffering from prostate, thyroid or mammary cancer, and it is evident that these factors certainly are absolutely independent of the surface tension. It is noteworthy that a lowered surface tension has been determined also in regenerating tissue,[41] and hence the connection of this physico-chemical property with growth processes appears substantiated. On the other hand, it cannot be considered as established that a decrease of the surface tension coïncides with the onset of tumor development, since an investigation of the cysticercus sarcoma of rats demonstrated a normal value for the surface tension in the primary tumor; a lowered surface tension was detected only in the subsequently transplanted tumors.[42] Inoculated rat tumors also exhibited a lowered surface tension, according to another study which employed the tensometric method,[43] but it seems very desirable for a clarification of this problem that pertinent investigation should include chemically induced animal tumors, permitting the following up of the relations between surface tension and tumor development.

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[33] E. Grafe & W. Rohmer: Deutsches Arch. f. klin. Med. *93*: 161; *94*: 239 (1908); *100*: 597 (1910).

[34] J. Fey & G. Lefmann: Med. Klin. *4*: 1751 (1908).

[35] J. Traube: Ztschr. f. Krebsforsch. *28*: 356 (1929).

[36] E. Bauer: Ztschr. f. Krebsforsch. *20*: 358 (1923).

[37] Z. Kagan: Ztschr. f. Krebsforsch. *21*: 155 (1924).

[38] B. Solowiew: Ztschr. f. Krebsforsch. *21*: 456 (1924).

[39] T. Kikuchi & G. Kawanishi: Jap. J. Obst. & Gynec. *18* : 340 (1935).

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[40] E. Bauet & A. Lasnitzki: Klin. Wchnschr. *4*: 395 (1925).

[41] T. Baturenko & L. Timofejewa: Ztschr. f. Krebsforsch. *37*: 436 (1932).

[42] I. P. Hennemann: Geneesk. tijdschr. v. Nederl.-Indie *72*: 1278 (1932); abstr. Am. J. Cancer *18*: 403 (1933).

[43] D. C. A. Butts, T. E. Huff & F. Palmer, Jr.: Science *65*: 304 (1927).

Numerous studies have been devoted to the determination of the surface tension in the blood serum of cancer patients, and a lowered surface tension (increased surface activity) in the prevailing majority of the cases has been observed.[44-53] These results were obtained by using both the tensometric and the stalagmometric methods, although the reliability of the latter method has been discounted when measurements of the surface tension are performed in protein-containing media.[54] The lowered surface tension of serum was not found to be confined to neoplasia, but may also be encountered in pregnancy[44] and in several non-malignant pathologies.[53] Some authors believed they had evidence for a decrease of the surface tension in the serum of cancer-free persons in proportion to advancing age,[45, 52] and saw in this an expression of a predisposition to cancer,[52] but the influence of age on the surface activity of serum has been contested.[55] Moreover, three incipient cases of cancer exhibited normal values of the surface tension of the serum,[56] and consequently the change of this physico-chemical quality obviously does not precede the tumor development. It was, however,

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44. B. M. Solowiew: Ztschr. f. Krebsforsch. *22*: 265 (1925).
45. F. Tománek: Časop. lék. česk. *69*: 570 (1930).
46. H. Güthmann & H. Frühauf: Arch. f. Gynäk. *134*: 425 (1928).
47. N. Louros & Gaessler: Ztschr. f. Krebsforsch *28*: 191 (1929).
48. P. S. Rabiner: Ztschr. f. Krebsforsch. *29*: Ref. 104 (1929).
49. W. Kopaczewski: Presse méd. *29*: 590 (1921); Compt. rend. Acad. d. sc. *179*: 1445 (1924); Ztschr. f. Krebsforsch. *27*: 273 (1928); Protoplasma *6*: 302 (1929).
50. A. H. Roffo & Minquenz: Prensa med. Argent. *2*: 5 (1917).
51. B. Maksimovič: Ž. Akuš. *44*: 281 (1933); abstr. Ztschr. f. Krebsforsch. *40*: Ref. 177 (1934).
52. A. Saitschenko: Biochem. Ztschr. *219*: 447 (1930).
53. O. Künzel: Ztschr. f. klin. Med. *133*: 607 (1938).
54. H. N. Harkins & W. D. Harkins: J. Clin. Investigation *7*: 263 (1929).
55. B. Kisch & O. Remertz: München. med. Wchnschr. *41*: 1097 (1914).
56. D. Adlersberg & E. Singer: Ztschr. f. d. ges. exper. Med. *46*: 500 (1925).

claimed that therapeutic measures, such as the administration of spleen preparations,[57] or—and this appears more convincing—effective tumor irradiation [53] caused the lowered surface tension of the serum to return to the normal. As to animal experiments, it was reported that inoculation of Rous sarcoma to fowl was soon followed by a decrease of the surface tension in the serum, and that analogous findings were obtained with spleen and liver extracts of the animals.[58, 59]

It may be of interest in connection with etiologic considerations that addition of several carcinogens to serum in vitro caused a lowering of the surface tension [60] and, similarly, the surface tension of serum against oil decreased after an admixture of dibenzanthracene.[61] Significant observations have been made in regard to the capacity of blood serum of buffering added surface-active substances: whereas normal serum is capable of absorbing small amounts of such substances, e.g., sodium glycocholate, without exhibiting a change of the surface tension, this capacity appeared markedly diminished in sera of cancer patients.[62] If sodium oleate was added to cancerous serum, and the surface tension repeatedly determined in certain time intervals, curves could be charted showing a characteristic difference from those obtained with serum of cancer-free persons tested under the same conditions; these studies, however, were not thought to have progressed far enough to permit the possibility of a diagnostic utilization of this phenomenon.[63] At any rate, it seems highly suggestive to connect these relations with the phenomena exhibited in the meiostagmin reaction, where changes of the surface tension of sera are measured following the addition of surface-active organ extracts (cf. Chapter X), especially in view of more recent studies, according to which addition of fatty acids—surface-active substances—to serum resulted in a different effect according to the patient from whom it was derived; that is, a subsequent ether treatment could not re-extract the fatty acids from serum of cancer-free persons, but it was able to do so in the case of sera of cancer patients.[64] Thus it may be inferred that in the latter instance the fatty acids are not bound by some serum constituents, as in normal serum and, therefore, their capillary activity is free to act by lowering the surface tension of the serum.

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[57] E. Bauer: München. med. Wchnschr. *72*: 1723 (1925).

[58] F. Herčik: Ztschr. f. Krebsforsch. *32*: 355 (1930).

[59] S. Kaneko: Tr. Jap. Path. Soc. *22*: 966 (1932).

[60] W.. Kopaczewski: Compt. rend. Soc. de biol. *118*: 1142 (1935).

[61] O. Reuterwall: Ztschr. f. Krebsforsch. *42*: 117 (1935).

[62] W. Kopaczewski: Compt. rend. Acad. d. sc. *191*: 1155 (1931).

[63] Ch. J. Keller & O. Künzel: Ztschr. f. klin. Med. *134*: 286 (1938).

Stalagmometric measurements seemed to indicate a lowered surface tension in the majority of the urines of cancer patients,[65, 66] but it is obvious that this deviation from the normal is neither regularly encountered in neoplasia, nor confined to this pathology, since many secondary factors may influence this physico-chemical quality of the urine, both physiologically and pathologically. One author believed he had detected a more specific reaction 'by comparing the surface activity of native and of acidified urine, inasmuch as urines of cancer patients should exhibit a more marked increase of the surface activity in response to the addition of acid than urines of cancer-free persons, and this rise of the "stalagmometric ratio" was considered as depending on the presence of increased amounts of protein decomposition

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64. M. Ascoli: Klin. Wchnschr. *14*: 1593 (1935).

65. J. Traube & F. Blumenthal: Ztschr. f. exper. Pathol. & Ther. *2*: 117 (1905).

66. A. J. Althausen, B. B. Varshavskaya & E. M. Krastina: Vopr. Onkol. *7*: 251 (1935): abstr, Ztschr. f. Krebsforsch. *44*: Ref. 252 (1936).

products.[67] This explanation, if valid, already makes it unlikely that the findings mentioned actually represent a characteristic quality of urines of cancer patients and, correspondingly, it has been found that the effect of acidification on urinary surface tension was not an appropriate means of differentiating urines of cancer-free and neoplastic patients.[68] Likewise, studies of the daily fluctuation of the surface tension, in urine specimens passed within twenty-four hours, did not demonstrate a behavior specific for cancer patients; these specimens showed a lesser degree of daily changes of the surface activity (isostalacturia) as compared with normal specimens. This phenomenon, however, was shared with other pathologies, such as consumptive tuberculosis, liver diseases and albuminuria, and it was correlated with the excretion of protein decomposition products.[69] More recently the adsorbability of the urinary surface-active substances has been tested; in the majority of urines of patients, afflicted with malignancy, a markedly decreased adsorbability of these substances to charcoal has been observed and similar relations were also noted in acute infections and tuberculosis. It is probable that these results indicated the prevalence of protein decomposition products in the urine of these patients, and it was surmised that the excretion of these substances might not only depend on tissue destruction, but be connected also with an impaired function of the reticulo-endothelial system present in the conditions named.[70]

Surface-active substances and the tumor process. Only very few, and not too convincing contributions of experimental nature have been made to the question whether, and in what direction, surface-active substances may influence the development and growth of malignant tumors. Findings have been mentioned above that in tissue cultures the growth of epithelial cells is promoted at the expense of mesenchymal elements following the addition of surface-active substances like cholesterol[14, 15] or bile,[71] and in Chapter II there was occasion to refer to a promotion of tumor growth occurring when the transplants had been treated with tributyrin previous to inoculation,[72] which effect may be connected with the surface activity of the ester tested. But, on the other hand, it has been reported that the tar cancer formation in mice was impeded by substances with strong capillary activity, such as by the intraperitoneal injection of oleic acid[73] or by the external application of sodium cholate.[74] These results, however, present difficulties for an adequate interpretation, inasmuch as it has by no means been proven that the effects observed really depended on the physico-chemical properties of the substances employed and since no actual determination of the surface tension in the treated tumors was performed. Therefore, it would appear very desirable to repeat such investigations under better defined experimental conditions.

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[67] W. Schemensky: Biochem. Ztschr. *105*: 229 (1920); Mitt. a. d. Grenzgeb. d. inn. Med. u. Chir. *34*: 451 (1922).

[68] K. Isaac-Krieger & W. Friedlaender: Klin. Wchnschr. *2*: 2171 (1923).

[69] D. Adlersberg & M. Sugár: Ztschr. f. d. ges. exper. Med. *46*: 466 (1925).

[70] K. Stern: Am. J. M. Sc. *202*: 229 (1941).

Adsorptive capacity of tumor tissue. The phenomenon of adsorption is definitely determined by the surface activity of the adsorbent, and hence is closely associated with the problem under discussion. Some experiments indicated a particularly great adsorbability of certain substances by tumors; in this respect it was claimed in older reports that carcinomatous cells exhibited in vitro a selective affinity for carbohydrates, whereas sarcomatous cells attracted peptones;

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71. M. Katzenstein & E. Knake: Ztschr. f. Krebsforsch. *33*: 378 (1931).
72. Z. Kagan: Ztschr. f. Krebsforsch. *21*: 453 (1924).
73. J. Lecloux: Compt. rend. Soc. de biol. *91*: 1155 (1924).
74. V. E. Mertens: Ztschr. f. Krebsforsch. *27*: 295 (1928).

these relations were interpreted as an especial avidity of the respective cells for the nutritive materials mentioned.[75] In the course of studies on the carcinolytic reaction (cf. Chapter X), it was observed that shaking of cancerous serum with cancer cells removed from the serum a factor responsible for the protection of cancer cells against the lytic action of normal serum,[76] and chemical analyses suggested a lipid nature of the factor adsorbed.[77] Since mouse cancer cells which had adsorbed the cancer-cell-protecting factor exhibited an increased virulence in subsequent inoculation,[78] a biologic significance can be attributed to this selective adsorption, possessed by neoplastic but not by normal cells. Regarding the mechanism of this adsorption, further information was gained in experiments which demonstrated that the lipids, extracted from malignant tumors, are capable of adsorbing the cancer-cell-protecting factor from the serum of cancer patients.[79] Hence the lipid structure of the cancer cells appears to be responsible for the increased, or selective adsorptive capacity of neoplasms, at least in the instance mentioned. If it is permissible to transfer these results, obtained in vitro, to the relationships in vivo, it may be assumed that the increased surface activity (lowered surface tension) of tumor extracts, described above, can be explained by an adsorption by the neoplasms of large amounts of surface-active substances from the surrounding body fluids.

While these adsorptive processes involve physiologic substances, numerous attempts have been made to prove a supposed selective affinity, viz., adsorption, of various inorganic and organic compounds by neoplastic tissue, but, as mentioned

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75. E. Freund & G. Kaminer: Biochem. Ztschr. *46*: 470 (1912).
76. R. Willheim & K. Stern: Wien. klin. Wchnschr. *43*: 227 (1930).
77. R. Willheim & K. Stern: Biochem. Ztschr. *226*: 315 (1930).
78. K. Stern & R. Willheim: Ztschr. f. Krebsforsch. *39*: 54 (1933).
79. R. Willheim & K. Stern: Biochem. Ztschr. *239*: 473 (1931).

in the preceding chapters, none of these claims could be unequivocally substantiated, and reports on a selective adsorption of copper sulfate and of copper seleniate by cancer cells in vitro[80] also cannot be considered as convincing, at least until further confirmation has been presented.

A special instance of selective adsorption is the phenomenon of vital staining, in which, however, conditions of cell permeability are also essentially involved. It is understandable that the discovery of a dyestuff, selectively staining neoplastic cells, would offer a very valuable diagnostic method, and might also be useful for therapeutic procedures, but the investigations made so far have not succeeded in this direction, nor do they give much reason for expecting such results in the future. Azo dyes were found to stain only the necrotic tumor parts, while the rest took up the dyes only after amounts had been administered which stained many normal organs[81, 82] and, likewise, injection of leuco bases into tumor-bearing animals resulted in a stain only of the necrotic tumor parts.[83, 84] The claim that acid triphenylmethane dyes (Fuchsin S, Isamine blue) were capable of vital staining of animal tumors[85] could not be corroborated insofar as isamine blue is concerned, which dye had been extensively used for therapeutic experiments but was on this occasion not seen to stain tumor cells. Evans blue (T 1824) has been injected intravenously into patients afflicted with neoplasms, but histologic examination showed a localization of the stain in fibroblasts, macrophages and exudate cells only and not in the tumor cells.[86] A review of the unsuccessful attempts selectively to localize dyes in malignant tumor cells was presented in 1931,[87] and investigations performed since this time essentially corroborated the earlier findings. It was reported that certain substituted naphthol sulfonic acids stained mouse carcinomas in vivo,[88] but no histologic analyses of these results, obtained with highly toxic compounds, were presented, nor have these observations been reexamined by other authors. Studies with Evans blue, a poorly diffusible azo dye, have been resumed more recently. In confirmation of the earlier publication, it was found that following intravenous injection of the dye into mice, rabbits and fowl, bearing transplanted or spontaneous tumors, the dye seemed to localize selectively around and in the neoplasms, but again it was noted that the staining affected the stroma only, while the tumor cells were not penetrated. A selective localization of analogous nature was also found after intravenous injection of foreign protein (animal serum), and these relations were explained by the assumption that the newly formed capillaries of the tumor and the tumor vicinity were more permeable to the dye and the proteins than the vessels of normal tissue.[89] This view appears supported by the fact that necrotic or sclerotic tumors or tumors, the vascular apparatus of which had been damaged by roentgen irradiation, did not exhibit this localization of the dye.[90] Another author found also acid azo dyes, injected into mice and rats with transplanted tumors, localized only in the phagocytes and in degenerating tumor cells.[91] Vital staining, with phenol red, of the skin of mice has been performed during the external application of tar, benzpyrene or dibenzanthracene; the changes observed—at first deeper red color of the treated skin than normal, then transformation into a yellowish tint—were supposed to be the expression of local vascular alterations, finally resulting in functional ischemia.[92] A different aspect is involved in the finding that, in tissue cultures of mouse sarcoma cells, living and dead cells may be differentiated by vital staining,[93] since under these conditions the cellular permeability alone determines the staining result.

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[80] A. H. Roffo & L. Correa: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *1*: 103 (1924).

[81] R. Weil: J. Cancer Res. *1*: 95 (1916).

[82] B. T. Simpson & M. C. Marsh: J. Cancer Res. *10*: 50 (1926); *11*: 417 (1927).

[83] L. Karczag, L. Teschler & G. Barok: Ztschr. f. Krebsforsch. *21*: 273 (1924).

[84] L. Karczag & L. Németh: Ztschr. f. Krebsforsch. *22*: 407 (1925).

[85] D. Engel: Ztschr. f. Krebsforsch. *22*: 365 (1925).

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[86] R. J. Ludford: Proc. Roy. Soc., London, s. B. *104*: 493 (1929).

[87] R. Cruickshank: Cancer Rev. *6*: 537 (1931).

[88] K. Kottmann: Schweiz. med. Wchnschr. *16*: 533 (1935).

[89] F. Duran-Reynals: Proc. Soc. Exper. Biol. & Med. *36*: 369 (1937); Am. J. Cancer *35*: 98 (1939).

[90] A. Brunschwig, R. L. Schmitz & T. H. Clarke: Arch. Path. *30*: 902 (1940).

[91] M. Hess: J. Path. & Bact. *51*: 309 (1940).

The absorptive capacity of serum has been tested by measuring the amount of ether which can be added without breaking up the ether-serum emulsion, and it was found that sera of cancer patients are able to bind up to eleven times the volume of ether, as compared with one to three volumes of ether absorbed by sera of cancer-free persons.[94] For this phenomenon, as well as for the observation that sera of cancer patients exhibit a greater tendency to emulsify fat,[95] changes of the lipid structure of the sera tested are most likely responsible.

3. *Actual Reaction (Hydrogen Ion Concentration).*

Hydrogen ion concentration of malignant tumors. The problem as to whether the hydrogen ion concentration in neoplasms deviates from that of normal organs in a characteristic way, and, if so, what change of this quality takes place, has been under intensive investigation, but the results obtained are of highly controversial nature. Although some workers believed themselves to be definitely entitled, on the basis of their experimental studies, to assume an alkaline reaction of tumors, it would be unwarranted to ignore the findings of several other exact investigations which led either to the opposite result or frequently indicated no specific alteration of the pH in neoplastic tissues. In analyzing these contradictory statements, it may be pointed out that the hydrogen ion concentration of living tissue undergoes continuous variations, depending on metabolic processes, and that the data gained by physico-chemical measurements represent at best a transient time differential, while at the same time it is problematic whether they can be regarded at all as physiologic values, and not merely as artifacts, due to disturbances caused by the experiments themselves. In this connection, it has even been contended that the application of the modern potentiometric methods to biologic substrates cannot always be considered as progress and as an advantage, since these extremely sensitive measurements may be influenced by processes not directly related to the value to be determined.[96] Furthermore, it must be remarked that most of the pertinent studies suffer from the disadvantage that reliable comparisons with normal values are lacking; for, if it still may be possible, in the case of human and spontaneous tumors of animals, to compare the respective results to figures obtained by analysis of the healthy parent tissues, such a procedure naturally is not given in the instances of inoculated tumors.

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[92] J. W. Orr: J. Path. & Bact. *44*: 19 (1937).

[93] M. Belkin & M. J. Shear: Am. J. Cancer *29*: 483; 499 (1937).

[94] M. Ascoli & R. Indovina: Klin. Wchnschr. *13*: 956 (1934).

[95] H. Eufinger & B. Schwemmler: Monatschr. f. Geburtsh. u. Gynäk. *97*: 533 (1934).

The following table (I), arranged in chronological manner, summarizes the results of numerous authors who determined the actual reaction in various malignant tumors.

Several supplementary remarks are appropriate for amplifying on some of the studies enumerated in the table, refer-

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[96] P. Rondoni: Cancro *4*: 203 (1933).

TABLE I.

Author	*Substrate*	*Result*
Woglom[97]	Flexner-Jobling carcinoma and Jensen sarcoma of rats	7.09–7.12 Normal range
Harde[98]	Rat carcinoma and sarcoma	
Harde & Danysz-Michel[99]	Mouse carcinoma and sarcoma	5.8–6.6 Acid reaction
Harde & Henry[100]	Mouse carcinoma and sarcoma	
Schmidtmann[100]	Human tumors, tar cancer of mice	5.8–6.7 Acid reaction
Millet[102]	Flexner-Jobling rat carcinoma	Average 6.8 (normal 7.1)
Milone[103]	Jensen rat sarcoma	Acid reaction
Goldfeder[104]	Ehrlich mouse carcinoma, mouse sarcoma, Rous sarcoma	7.5–7.7 Alkaline reaction
Kobliha[105]	Rous fowl sarcoma	Center: 7.01–7.50 Periphery: 6.48–7.00
Joos[106]	Human and animal tumors	7.15–7.70 Alkaline reaction
Chambers & Ludford[107]	Tissue cultures of human breast cancer and of mouse sarcoma	Normal (no difference from normal tissue cultures)

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97. W. H. Woglom: J. Cancer Res *8*: 34 (1924).
98. E. Harde: Compt. rend. Soc. de biol. *94*: 1346 (1926).
99. E. Harde & M Danysz-Michel: Compt. rend. Soc. de biol. *95*: 1489 (1926).
100. E. Harde & P. Henry: Ann. Inst. Pasteur *41*: 1022 (1926).
101. M. Schmidtmann: Ztschr. f. d. ges. exper. Med. *57*: 127 (1927).
102. H. J. Millet: J. Biol. Chem. *78*: 281 (1928).
103. S. Milone: Arch. per le sc. med. *52*: 21 (1928), abstr. Cancer Rev. *7*: 496 (1932).
104. A. Goldfeder: Ztschr. f. Krebsforsch. *29*: 134 (1929).
105. F. Kobliha: Ztschr. f. Krebsforsch. *32*: 544 (1930).
106. G. Joos: Frankfurt. Ztschr. f. Path *39*: 254 (1930).
107. R. Chambers & R. J. Ludford: Proc. Roy Soc., London, s. B, *110*: 120 (1932).

Author	*Substrate*	*Result*
Voegtlin & Kahler[108]	Rous sarcoma (intramuscular)	6.32 Acid reaction
	Rous sarcoma (subcutaneous)	6.78–6.82 Acid reaction
	Walker rat carcinoma	Slightly acid reaction
	Jensen rat sarcoma	
Okuneff[109]	Human and mouse tumors	6.0–7.56 Average: 6.6 Acid reaction
Goldfeder, Partridge & Bowles[110, 111]	Mouse tumors	Average: 7.36 Alkaline reaction
Moratti[112]	Human skin cancer	Normal
Benetato[113]	Rat tumors	7.11–7.40 Alkaline reaction (muscle of adult animal: 6.95–7.12; muscle of newborn: 7.08–7.12; muscle of embryo: 7.33–7.44)
Seeger[114]	Ehrlich ascites carcinoma of mouse	6.0–8.4 Alkaline reaction

ring to the technique used and the interpretation offered by the authors themselves. In the first investigation mentioned,[97] freshly prepared, filtered extracts of the tumors were tested by a colorimetric method, while injections of indicator substances into tumor-bearing animals have been employed in other studies.[98–100] On the other hand, electrometric determinations of the pH were performed by many other authors, either employing quinhydrone electrodes[104,105,109] or glass electrodes.[102,108,110,111] Taking into consideration, moreover, that the experimental conditions varied widely, inasmuch as some of the measurements were applied to removed tumor material in vitro,[101,102,104,105,109,110,111] to living, anesthetized animals,[108] or to tissue cultures,[107,114] it must be conceded that uniform results could hardly be expected, especially in view of the great variety of human and animal tumors tested. As has been shown, even the same type of tumor—Rous sarcoma—may exhibit markedly differing values of the pH, depending on whether subcutaneous or intramuscular inoculation is used.[108] Nevertheless, some authors[104,106,110,111,113] considered their findings as definitely indicating a shift of the tumor reaction to the alkaline side, and if an attempt is made to reconcile such a view with the opposing results obtained in other investigations,[97,102,103,108] in addition to the factors mentioned above, some other circumstances must be allowed for as influencing the pH of neoplasms. Thus a study with the Rous sarcoma showed that immediately following the tumor inoculation—but also after injection of ground muscle—a more acid reaction became apparent at the site of the injection, which was considered as a sign of inflammation, and that the developing sarcoma gradually assumed a more alkaline reaction, exhibiting after six days a pH of 6.99 and after twenty-one days a pH value of 7.35.[105] Determinations of the pH in Ehrlich mouse carcinomas and in human breast cancers yielded values ranging from 6.0 to 7.56, and the following factors were recognized as causing these great variations. Young tumors were more acid than older ones, the tumor periphery more acid than the central parts, and cell-rich tumors more acid than scirrhous, cell-poor ones.[109] Therapeutic measures may influence the actual reaction of tumors, at least as far as roentgen or radium administration are concerned, since these treatments were found to exert an acidifying effect on the tumor reaction.[115] Finally, attention has been called to the great significance of the buffering capacity inherent in neoplastic tissue,[104, 109] by virtue of which property the tumors may prevent the lactic acid, formed in large amounts in the course of their carbohydrate metabolism, from affecting the actual reaction An increased buffering capacity for acids has indeed been ascertained in malignant tumors, in which bicarbonates, potassium protein salts and inorganic phosphates were supposed to act as such buffering substances,[116] and the buffering capacity of rat tumors was reported to exceed that of rat embryos, although both tissues exhibited approximately the same degree of alkalinity.[113] These relations have been used for an explanation as to how the seemingly paradoxical finding of an alkaline reaction might accompany the excessive glycolytic capacity of malignant tumors, i.e., a formation of large amounts of acid valences in the form of lactic acid. In view of the increased amount of buffers, it was assumed that these liberated hydrogen ions would not only be bound in the neoplasms, but an over-compensation might also lead to an alkaline pH. It must, however, be pointed out that these hypothetical assumptions do not appear wholly supported by experimental results obtained with tissue cultures. Here neoplastic cells were found to increase the acidity of the culture medium to a much higher degree than that of normal cells,[117] obviously on account of the excessive formation of lactic acid. Likewise, parenteral administration of various sugars to tumor-bearing animals (Jensen rat sarcoma, Flexner-Jobling rat carcinoma, Walker rat sarcoma, spontaneous mouse tumors) was followed by a considerable decrease of the pH of the tumors,

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[108] C. Voegtlin & H. Kahler: Science *75*: 362 (1932).

[109] N. Okuneff: Ztschr. f. Krebsforsch. *38*: 283 (1933).

[110] A. Goldfeder, H. M. Partridge & J. A. C. Bowles: Ztschr. f. Krebsforsch. *40*: 186 (1933).

[111] H. M. Partridge, J. A. C. Bowles & A. Goldfeder: J. Lab. & Clin. Med. *19*: 1100 (1934).

[112] A. Moratti: Tumori n. s. *8*: 220 (1934).

[113] G. Benetato: Compt. rend. Soc. de biol. *116*: 1093 (1934).

[114] P. G. Seeger: Arch. f. exper. Zellforsch. *22*: 356 (1939).

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115. A. Goldfeder: Ztschr. f. Krebsforsch. *38*: 135; 142 (1932).

116. N. Okuneff & O. Tschistowitch: Ztschr. f. Krebsforsch. *38*: 178 (1932).

117. A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *7*: 239 (1931).

measured in vivo by means of glass electrodes (e.g., a drop from 7.07, before the sugar injection, to 6.3 after this procedure was observed). This effect was viewed as the consequence of increased glycolysis, but since pH-determinations in the vicinity of the tumor did not indicate appreciable changes following the sugar administration, it was concluded that the lactic acid formed did not penetrate the surrounding tissue, and, for this reason, earlier theories, holding lactic acid responsible for the invasive tumor growth (cf. Chapter II), were rejected.[118] It was, however, proven experimentally that the shift to acidosis, occurring in consequence of increased glycolysis, is of great significance for the neoplastic process, inasmuch as this acid medium favors protein synthesis in the tumors.[119]

Summarizing the problem under discussion, it may be stated that the experimental material available furnished no evidence for an assumption that the formation of tumors depended on the occurrence of a specific change of the actual reaction, and the fact that measurements of the pH in various neoplasms led to divergent results, apart from differences in the technique employed, may be due to secondary factors. There seems to be a tendency to alkalosis running parallel to the aging and to the advancing necrosis of the tumors; increased amounts of buffering substances in the neoplasms may conceal, or even over-compensate changes of the actual reaction conditioned by the tumor metabolism; on the other hand, experimental evidence indicates that these metabolic processes, especially glycolysis, may decisively influence the pH, measured in tumors at a certain moment.

Hydrogen ion concentration of the blood. Theoretically,

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118. C. Voegtlin, R. H. Fitch, H. Kahler, J. M. Johnson & J. W. Thompson: Nat. Inst. Health Bull. No. 164, 1 (1935).

119. M. E. Maver, J. M. Johnson & C. Voegtlin: Nat. Inst. Health Bull. No. 164, 29 (1935).

one should expect to encounter much better defined relations regarding the pH of the blood, since it is known that this medium possesses a strong tendency to retain its original chemical and physico-chemical qualities. But at the same time blood fulfills the task of maintaining the normal state of the body tissues, by removing from them all those products of deterioration or of abnormal metabolism, which threaten to disturb the balance essential for the integrity of vital functions. For these reasons, studies of the blood reaction may evoke a greater interest than those dealing with the actual reaction of the neoplasms themselves, since the finding of a definite deviation from normal may be interpreted as an indication of a systemic change of the pH which may cause, accompany, or result from neoplasia. The results of the exceedingly numerous investigations carried out regarding this problem are again compiled in a table (II), and the results obtained, as well as etiologic or therapeutic conclusions derived from them, will be discussed in connection with this survey.

Although the results enumerated are far from uniform, it will be noted that findings of an alkalosis have been most frequently registered in these investigations. But, at the same time, one does not appear justified in attributing the divergent statements recorded wholly to differences of the tested material, or of the methods employed. It has been emphasized by Reding,[152] one of the staunchest supporters of an essential connection between neoplasia and blood alkalosis, that the greatest care must be taken in securing reliable results of the pH determination in blood, by avoiding atmospheric contaminations of the samples, by special cleaning procedures applied to the equipment which should be used exclusively for this purpose, and by adequately preparing the persons tested prior to withdrawal of the blood specimen. When all these precautionary measures were observed, the author ob-

TABLE II.

Authors	*Substrate*	*Result*
Moore & Wilson[120]	Human serum	Alkalosis
Sturrock[121]	Human serum	Alkalosis
Waterman[122]	Human plasma	Alkalosis
Chambers[123]	Dialysate of human blood	Alkalosis 7.45
Myers, Schmitz & Booher[124]	Human blood	Normal
Corran & Lewis[125]	Human blood	Normal
Roffo & Correa[126]	Human serum	Alkalosis, progressing with tumor growth
Laclau & Rabinovitch[127]	Rat blood	Normal
Peyre & Sannié[128]	Human blood	no characteristic change
McDonald[129]	Human plasma	Alkalosis
Fischer[130]	Human plasma; human serum, separated after 24 hours	Normal Acidosis

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120. B. Moore & F. P. Wilson: Biochem. J. *1*: 297 (1906).

121. W. D. Sturrock: Brit. M. J. *2*: 780 (1913).

122. N. Waterman: Biochem. Ztschr. *133*: 535 (1922).

123. W. H. Chambers: J. Biol. Chem. *55*: 229; 257 (1923).

124. V. C. Myers, H. W. Schmitz & L. E. Booher: J. Biol. Chem. *57*: 209 (1923).

125. J. W. Corran & W. C. M. Lewis: Biochem. J. *18*: 1358 (1924).

126. A. H. Roffo & L. Correa: Bol. inst. med exper. para el estud. y trat. d. cáncer *1*: 111 (1924).

127. N. Laclau & R. Rabinovitch: Rev. Soc. Argent. Biol. *1*: 635 (1925); Compt. rend. Soc. de biol. *93*: 1635 (1925).

128. E. Peyre & C. Sannié: Bull. Assoc. franc. p. l'étude du cancer *14*: 350 (1925).

129. E. McDonald: Med. J. & Rec. *125*: 795 (1927).

130. R. Fischer; quot. R. Fischer & A. Kotzareff: Les Cancers et la Physicochimie, Vigot frères, Paris 1927.

Authors	*Substrate*	*Result*
Sendrail[131]	Blood of rabbits with tar cancer	Acidosis
Váňa[132]	Human plasma	Alkalosis
Weiss, Sumegi & Udvardi[133]	Human plasma	Alkalosis, average 7.66
Schneider & Achelis[134]	Human plasma	Acidosis, 7.25–7.44
Louros & Gaessler[135]	Human plasma	Acidosis, 7.25–7.44
Millet[136]	Human serum	Normal
Schreus[137]	Human blood	Slight alkalosis, 7.43 (normal 7.38)
Guthmann & Wirz[138]	Human blood	Incipient stages: acidosis; later, alkalosis
Wirz[139]	Human blood	Incipient stages: acidosis; later, alkalosis
Jaumain[140]	Human plasma	Alkalosis
Bischoff, Long & Hill[141]	Human plasma	Slight alkalosis, 7.50 (normal 7.46)
Beregoff[142]	Human plasma	Alkalosis

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131. Sendrail: Thèse de Toulon (1925); quot. Fischer & Kotzareff, loc. cit. 130.

132. A. Váňa: Mitt. a. d. Grenzgeb. d. Med. u. Chir. *40*: 514 (1927).

133. S. Weiss, S. Sumegi & L. Udvardy: Klin. Wchnschr. *7*: 1178 (1928).

134. E. Schneider & H. Achelis: Klin. Wchnschr. *7*: 1955 (1928).

135. N. Louros & Gaessler: Ztschr. f. Krebsforsch. *28*: 191 (1929).

136. H. J. Millet: J. Biol. Chem. *82*: 263 (1929).

137. H. Schreus: Klin. Wchnschr. *8*: 1002 (1929); Strahlentherapie *33*: 243 (1929).

138. H. Guthmann & P. Wirz: Arch. f. Gynak. *142*: 530 (1930).

139. P. Wirz: Klin. Wchnschr. *9*: 1102 (1930).

140. D. Jaumain: Compt. rend. Soc. de biol. *103*. 947 (1930).

141. F. Bischoff, M. L. Long & E. Hill: J. Pharmacol. & Exper. Ther. *39*: 425 (1930).

142. P. Beregoff; J. Cancer Res. *14*: 559 (1930).

Authors	*Substrate*	*Result*
Woodard[143]	Human plasma	no characteristic change
Mercier & Rossier[144]	Human plasma	Slight alkalosis, 7.36 (normal 7.33)
Woodward, Schoonover, Fry & McDonald[145]	Human plasma	Alkalosis: 7.44 (normal 7.35–7.40)
Baumann[146]	Human blood, arterialized	Alkalosis
Hueper, Woodward & Fry[147]	Human plasma	Alkalosis (increasing parallel to malignancy)
Kobiiha[105]	Blood of fowl with Rous sarcoma	No characteristic change; ante exitum: Alkalosis
Bugnard, Colombies, Miletsky & Boursiac[148]	Human plasma	Alkalosis
Slosse & Reding[149]	Human plasma	Alkalosis: 7.48; normal 7.36
Des Ligneris[150]	Blood of fowl with tumors	First stages, alkalosis; later, acidosis
Pentimalli[151]	Blood of fowl with Rous sarcoma	Acidosis, increasing parallel to tumor growth

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143. H. Q. Woodard: J. Cancer Res. *14*: 319 (1930).

144. P. Mercier & P. H. Rossier: Cancer, Bruxelles *7*: 249 (1930).

145. G. E. Woodward, J. W. Schoonover, E. G. Fry, E. G. Torrance & E. McDonald: J. Lab & Clin. Med. *16*: 704 (1931).

146. J. Baumann: Deutsche Ztschr. f. Chir. *234*: 56 (1931).

147. W. C. Hueper, G. E. Woodward & E. G. Fry: Am. J. Cancer *15*: 2666 (1931).

148. L. Bugnard, F. H. Colombies, Q. Miletzky & R. Boursiac: Bull. Assoc. franç. p. l'étude du cancer *20*: 596 (1931).

149. A. Slosse & R. Reding; quot. R. Reding: Le terrain cancereux et cancérisable, Masson, Paris 1932.

150. M. J. A. Des Ligneris: Brit. J. Exper. Path *13*: 189 (1932).

151. F. Pentimalli: Ztschr. f. Krebsforsch. *37*: 10 (1932).

Authors	*Substrate*	*Result*
Reding[152]	Human plasma	Alkalosis: 7.38–7.44
Fujimaki, Arimoto, Nomura, Kimura & Tani[153]	Plasma of rabbits with sarcoma	Alkalosis (increasing with tumor advancement)
Ely[154]	Human blood	no characteristic change
Benetato & Benetato-Modval[155]	Human plasma	7.21–7.52 (normal: 7.32–7.36); 20% of cancer patients below normal, 30% above normal
Dickinson & Havard[156]	Human blood from radial artery	no characteristic change
Lacassagne, Loiseleur & Nyka[157]	Blood of rabbits with Brown-Pearce carcinoma	uncharacteristic oscillations
Selvaggi[158]	Human blood	Alkalosis; more pronounced in adenocarcinoma than in skin cancer
Collier[159]	Plasma of rabbits with Brown-Pearce carcinoma	Normal
Lasch & Lustig[160]	Blood of rabbits with Brown-Pearce carcinoma	Normal

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152. R. Reding: Le terrain cancereux et cancérisable, Masson, Paris 1932.

153. Y. Fujimaki, K. Arimoto, R. Nomura, T. Kimura & I. Tani: Tr. Jap. Path. Soc. *22*: 971 (1933).

154. J. O. Ely: Am. J. Cancer *17*: 58 (1933).

155. G. Benetato & M. Benetato-Modval: Compt. rend. Soc. de biol. *114*: 329 (1933).

156. S. Dickinson & R. E. Havard: Brit. J. Exper. Path. *14*: 394 (1933).

157. A. Lacassagne, J. Loiseleur & W. Nyka: Compt. rend. Soc. de biol. *113*: 1379 (1933).

158. G. Selvaggi: Tumori n. s. *8*: 393 (1934).

159. W. A. Collier: Ztschr. f. Krebsforsch. *40*: 585 (1934).

160. F. Lasch & B. Lustig: Ztschr. f. Krebsforsch. *43*: 146 (1936).

Authors	*Substrate*	*Result*
Aisenberg, Krizschin, Gitis & Erusalimski[161]	Human blood	Alkalosis in advanced tumor cases
Kageyama[162]	Human blood (uterine cancer)	Slight alkalosis
	Blood of sarcoma-bearing rabbits	Alkalosis, increasing with tumor advancement
Colombo[163]	Human arterial blood	Normal
Kowalczykowa[164]	Blood of rabbits with tar cancer	Normal; in later stages, acidosis
Oszacki & Kurzweil[165]	Human blood	Alkalosis
Gasinski[166]	Human blood	Alkalosis
Eldahl[167]	Human blood	Normal; in cachexia only, alkalosis

tained a far reaching agreement between the results of colorimetric and of potentiometric pH measurements performed in the same blood specimen. But, furthermore, a careful selection of the patients tested was considered of paramount importance, inasmuch as herewith secondary factors, influencing the reaction, but not directly connected with the tumor process, should be excluded as much as possible. It is easily comprehensible that, in patients suffering from advanced neo-

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161. S. I. Aisenberg, D. G. Krizschin, D. O. Gitis & L. M. Erusalimski: Med. Ž. vševkrain nauk *4*: 1147 (1935); abstr. Ztschr. f. Krebsforsch. *44*: Ref. 68 (1936).

162. T. Kageyama: Jap. J. Obst. & Gynec. *19*: 570 (1936).

163. C. Colombo: Arch. ital. chir. *43*: 392 (1936); abstr. Ztschr. f. Krebsforsch. *45*: Ref. 253 (1937).

164. J. Kowalczykowa: Bull. internat. Acad. pol. sc., cl. méd., No. 3, 4, 141 (1936); abstr. Ztschr. f. Krebsforsch. *45*: Ref. 321 (1937).

165. A. Oszacki & R. Kurzweil: Biochem. Ztschr. *289*: 234 (1937).

166. J. Gasinski: Arch. f. klin. Chir. *190*: 73 (1937).

167. A. Eldahl: Nord. Med. (Stockholm) 1939: 2938; abstr. Ztschr. f. Krebsforsch. *50*: Ref. 329 (1940).

plasms and exhibiting a progressive cachexia, the increased tissue destruction and the subsequent excessive liberation of protein decomposition products, or severe nutritional disturbances and secondary infections, may lead to alterations of the pH of the blood. Apart from these cases, Reding also excluded patients afflicted with tumors of the respiratory tract (on account of the possibility of carbon dioxide-accumulation in the blood), or with gastric neoplasms (on account of the possibility of acidosis in pyloric stenosis, and alkalosis following frequent vomiting), and cases accompanied by severe hemorrhages, since this occurrence was found to result in acidosis.[168-170] Finally, therapeutic measures, it was stated, must be allowed for, especially regarding radiation therapy which it was observed shifted the blood reaction to the acid side.[cf. 116, 169] By selecting the tested cases according to these lines, Reding and a number of other authors encountered with great regularity an alkalosis of the blood in cancer patients, and they explained opposing findings as being due to neglect of the precautionary measures enumerated. But it is obvious that, even if the change of the blood reaction in neoplasia may be regarded as a specific phenomenon, the possibility of its diagnostic utilization is not given, both on account of the necessary limitation to selected tumor patients, and also because of the delicate equipment and complex working conditions required, which generally cannot be realized in clinical studies.

Turning to the investigations of the pH of the blood in neoplasia, which led to divergent results, some relations must be discussed which may clarify these questions. It is only of historical interest to refer to work done prior to the estab-

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168. M. A. Bennett: J. Biol. Chem. *69*: 675 (1926).
169. F. W. Hartman & R. Gesell: Am. J. Physiol. *81*: 650 (1927).
170. C. G. Johnston & D. W. Wilson: J. Biol. Chem. *85*: 727 (1930).

lishment of the conception of hydrogen ion concentration and to the development of methods for its determination; at that time, the alkalinity of cancer serum was measured by titration, and a decrease of this titration alkalinity was claimed as occurring in the serum of cancer patients,[171–173] although this finding was not regularly encountered by one author.[174] Later studies, however, led to the opposite results, i.e., of an increased alkalinity of cancer serum, as measured by titration, and this deviation from the normal was declared to depend on the protein fractions, since serum ashes of normal or of cancer-free persons did not exhibit any difference in this respect.[120, 121] It is, therefore, suggested that these findings should be connected to an increase of the acid-binding valences of the serum proteins in neoplasia, and therefore these observations are rather connected with the buffering capacity than with the actual reaction. As to investigations performed with adequate modern methods, it has been contended that only the use of whole blood would warrant reliable results[165] and, from another side, it was pointed out that changes of the pH may appear in the venous blood, whereas arterial blood of the same patient exhibited normal conditions.[163]

Naturally, the explanations offered for changes of the blood pH in cancer, and the significance attributed to them, varied according to the results obtained by the individual authors Those finding an acidosis regarded this phenomenon as a secondary consequence of the neoplastic process, such as accumulation of fatty acids,[131] or as an augmentation of lactic acid[151] in the blood On the other hand, several authors

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171. R. von Jaksch: Ztschr. f klin Med *13*: 420 (1888).
172. H. Rumpf. Zentralbl f. klin. Med 1891: 441
173. F. Klemperer: Verhandl. d. Congr. f. inn. Med, Vienna 1890.
174. H. Strauss: Ztschr. f. klin. Med. *30*. 317 (1896)

who believed they had evidence for an alkalosis of the cancer blood [152, 175] viewed this phenomenon as an expression of a predisposition to neoplasia, assigning to it a great significance for the tumor development. As supporting this conception, they pointed to the observation that apparently healthy members of families, exhibiting a hereditary tendency to tumor formation, showed a slight alkalosis of their blood, and that surgical tumor removal was not followed by a reversion of the pH to the normal.[152] The validity of this theory, however, must be strongly questioned, not only because research workers contested the latter claim and found normal pH values after radical operation of malignant tumors,[165, 166, 176] but mainly because it is apparent from several studies that the development of alkalosis does not precede the tumor formation, but it increases parallel to the advancing neoplastic growth [105, 126, 138, 162, 165–167] and, moreover, animal experiments clearly indicated that unimpaired growth of inoculated or chemically induced tumors may be accompanied by an acidosis of the blood.[131, 150, 151, 164] Taking into consideration, furthermore, the numerous reports of normal pH values found in the blood of cancer patients,[124, 125, 163, 167] or of tumor animals, [105, 127, 159, 160] it is not to be doubted that, in the course of neoplastic diseases, an alkalosis of the blood may very frequently arise; nevertheless this phenomenon cannot be accepted as representing a necessary factor for predisposition to neoplasms or for their growth. Under these circumstances, the validity of another theory, which envisaged a difference of the pH between the cancer cell and between the surrounding humoral or cellular medium as a decisive factor for tumor development, must be questioned, in spite of the originality of the idea. The conception was incited by the

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175. W. Meyer: Cancer; its origin, its development and its self-perpetuation, Hoeber, New York 1931.

176. A. Oszacki: 1st Internat. Cancer Congress, Madrid *2*:73 (1933).

observation that during the process of the fetal nidation a difference between the pH of the fertilized ovum and of the uterine mucosa became apparent.[177] As stated in the preceding section, no convincing evidence has been furnished to demonstrate the existence of a regular pH-differential between incipient tumors and their surroundings, nor has the present survey indicated such a phenomenon as to the humoral media.

Attempts have been made to correlate the anacidity or hypochlorhydria of the gastric contents in patients suffering from gastric cancer, as well as in patients afflicted with tumors of other organs, with the alleged alkalosis of the blood.[178, 179] The apparent weakness of this conception lies in the fact that neither the blood alkalosis nor the achlorhydria, as far as extragastric tumors are concerned, are regularly associated with malignant tumors. In the latter respect it is true, absence of or very low values of hydrochloric acid have been observed by some authors [180–183] in patients suffering from cancers of the female genital tract or from other extragastric tumors, but other workers found normal gastric acid values in extragastric malignancy,[184] or obtained low figures in extragastric abdominal tumors only in the presence of large liver metastases.[185] A survey on several pertinent investigations has been given, and it was declared that hypochlorhydria, when encountered in patients with extragastric malignancy, may be simply due to the frequency of these diseases in advanced age, where a tendency to decreased secretion of hydrochloric acid generally prevails; experiments with rabbits inoculated with Brown-Pearce carcinoma did not indicate that the tumor transplantation and growth had any effect on the gastric secretion.[186] Interesting findings have been reported in studies in which the injection of extracts of achlorhydric cancerous stomachs—and similarly of stomach extracts of patients with pernicious anemia—into dogs inhibited the gastric secretion of the animals. It was tentatively assumed that in these stomachs a gastric secretory depressant was present and, in the case of gastric cancer, may precede the tumor formation. But since similar results were also obtained with extracts of some normal stomachs, and these investigations have not been as yet concluded,[187, 188] a final evaluation of these observations must await a continuation of the experiments.

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[177] R. Fischer & A. Kotzareff: Les Cancers et la Physicochimie, Vigot frères, Paris 1927, pp. 151, 171 seq.

[178] B. Moore, W. Alexander, R. E. Kelly & H. E. Roaf: Lancet *1*: 1120 (1905).

[179] F. W. M. Palmer: Biochem. J. *1*: 398 (1906).

[180] J. Friedenwald & L. T. Brown: Med. J. & Rec. *126*: 491 (1927).

[181] K. Fuge: Monatschr. f. Geburtsh. u. Gynäk. *97*: 37 (1934).

[182] E. Nekula: Zentralbl. f. Gynäk. 1938, No. 42.

[183] H. Yazima: Okayama-Igakkai-Zasshi *48*: 638 (1936); abstr. Ztschr. f. Krebsforsch. *45*: Ref. 37 (1937).

[184] B. Dahl: Acta med. Scandinav. *81*: 565 (1934).

[185] A. Bertola: Gior. di clin. med. *16*: 1427 (1935).

Controversial statements which have been made as to the actual reaction of the urine of cancer patients are not surprising, insofar as this quality is influenced by several secondary factors, such as nutrition, renal function, and decomposition of proteins. Consequently, the reports of some authors, claiming an increased alkalinity of urines in neoplasia,[189, 190] have been either limited by other workers to intestinal tumors (impaired resorption of food),[191] or have been altogether contested.[192] An exact study has been

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186. H. Necheles & M. Appel: Am J. Digest. Dis. *6*: 261 (1939).

187. A. Brunschwig, T. H. Clarke, J. van Prohaska & R L Schmitz· Surg., Gynec. & Obst *70*: 25 (1940).

188. A. Brunschwig, R. L. Schmitz & R. Rasmussen: J Nat. Cancer Inst. *1*: 481 (1941).

189. N. Waterman: Ergebn. d. inn Med. u. Kinderh *30*: 304 (1926).

190. P. Delbet & Franicevic: Tribune méd. *67* · 1 (1933); abstr Am J. Cancer *21*: 878 (1934).

191. R. Dupont & Dallongeville · Bull. Assoc. franç. p. l'étude du cancer *21*: 574 (1932).

192. C. Rodriguez. Arch. españ. oncol. *3*: 293 (1933).

performed with the urines of one hundred patients suffering mostly from cancers of the oral cavity, of glands of skin, or of the breast while receiving a well controlled diet The results obtained gave no indication of a urinary alkalinity; on the contrary, a marked increase of the excretion of ammonium salts was found.[193] This phenomenon is known to be associated with acidosis, in which condition ammonia, instead of fixed bases, is used for neutralizing acids eliminated through the kidneys.

Experimental changes of the hydrogen ion concentration. There can be no doubt as to the decisive influence on metabolic and growth processes, which may be exerted by even slight changes of the actual reaction and, accordingly, pertinent investigations relating to malignant tumors appeared justified. It was observed that the growth of embryonic heart cells in tissue cultures was enhanced by increasing the alkalinity of the medium,[194] and neoplastic explants were found to be more sensitive than normal cells to the addition of acid but they tolerated a shift to the alkaline side better than normal cells.[195] The results obtained with animal tumors, however, did not exhibit a uniform aspect. Filtrates of the Rous fowl sarcoma brought to a pH of 7.5, were seen to produce slowly growing, small tumors only, whereas injections of filtrates of a pH of 5.5 resulted in rapidly growing tumors.[98] Other authors claimed that the transplantation of mouse carcinomas in alkaline solutions (0.1 per cent of sodium bicarbonate) promoted subsequent tumor growth, while acid solutions (addition of hydrochloric acid, nitric acid, suberic acid or succinic acid) caused a retardation of the neoplastic growth.[196] Finally, it was reported that successful

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193. E. Silvestroni: Tumori n. s. *11*: 442 (1937); *12*: 1 (1938).
194. J. Manger & A. Hock: Biochem. Ztschr. *254*: 176 (1932).
195. A. Fischer: Ztschr. f. Krebsforsch. *26*: 256 (1932).
196. J. Flaszen & H. Wachtel: Klin. Wchnschr. *8*: 1912 (1929).

inoculations of mouse carcinomas could be performed with tumor material which had been treated for twenty-four hours with buffer solutions (glycine-sodium hydroxide-hydrochloric acid), ranging in their pH from 3.68 to 9.71, without recognizable differences in the subsequent tumor growth.[159] Consequently, these experiments did not indicate a regular and generally valid influence of the hydrogen ion concentration on the growth of inoculated tumors but, nevertheless, several other experimental arrangements have been proposed for the purpose of eliciting changes of the pH in the organism, which alterations might affect malignant tumors. A conception, which will be referred to in detail in connection with nutrition, was based on the claim that an alkaline diet was able to induce spontaneous animal tumors.[197] No confirmation of this finding has been recorded, but it has been reported that a predominantly alkaline diet enhances the growth of inoculated tumors [198] and, correspondingly, attempts have been made to inhibit the tumor growth by causing an acidosis in the animal body. Some success has been seen in an acidifying therapy, which consists of peroral administration of calcium chloride and ammonium chloride, together with the injection of monoiodo-acetic acid, inasmuch as mouse and rat tumors were cured by this treatment in twenty per cent of the animals, or retarded in their growth in the rest.[199] Although, doubtless, the halogen acetic acid was essentially involved in this effect, the significance of the acidification seemed proven by the finding of an increased hydrogen ion concentration in the tumors and organs of the treated animals. Other experiments, however, were less successful. Peroral administration of acids or alkalis, or an acidogenic diet, did not affect the

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197. O. L. E. deRaadt: Ztschr. f. Krebsforsch. *32*: 596 (1930).
198. A. Goldfeder: Ztschr. f. Krebsforsch. *38*: 121 (1932).
199. A. Goldfeder: Ztschr. f. Krebsforsch. *39*: 417; 421 (1933).

growth of mouse carcinomas,[191, 200] of Rous sarcoma,[201] or of rabbit sarcoma,[202] and similar negative results have been obtained in clinical experiments with acidifying drugs.[189, 191] Even the forcible attempt to change the actual reaction of mouse tumors by direct iontophoresis of hydrogen ions led only to local necrosis, but never to complete tumor regression.[203] Another approach to provoking acidosis in cancer patients for therapeutic purposes, by means of the inhalation of oxygen-carbon dioxide mixtures,[204] will be discussed in the chapter on metabolism, and the theoretical foundation and the clinical results of this method will also be presented there.

Finally, it must be mentioned that sarcoma formation has been observed in mice which had received repeated injections of 0.5 per cent hydrochloric acid, buffered with phthalate at a pH of 5.0, for eight to eleven months; the tumors were produced at or near the site of the injections. It was thought unlikely that the phthalate possessed a carcinogenic activity, but these findings were considered as proof that comparatively simple chemical or physico-chemical stimuli, if applied for a long enough period, might induce tumor development and, in this respect, the sarcoma was believed to represent a less specific response than carcinoma.[205]

4. *Oxidation-Reduction Potential.*

The significance for the cancer problem of this physicochemical quality permitting an insight into the processes of

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[200] P. Rondoni: Rendiconti R. acad. lincei *17*: No. 11 (1933).

[201] G. Guazzieri: Riv. di pat. sper. *1*: 347 (1934).

[202] H. Sakaino: abstr. Ztschr. f. Krebsforsch. *43*: Ref. 127 (1936).

[203] N. Okuneff: Acta cancrol. *1*: 357 (1935).

[204] B. Fischer-Wasels: Gastherapie bösartiger Geschwülste, Frankfurt a. M., 1929.

[205] V. Suntzeff, R. S. Babcock & L. Loeb: Am. J. Cancer *39*: 56 (1940).

oxidation, which take place in every cell and every tissue, is greatly intensified by the fact that tumor metabolism, as discussed in the chapter on metabolism, exhibits very marked deviations from the normal, and these alterations may be presupposed to lead to an accumulation in the neoplasms and in the affected organism of oxidisable substances which would exert a negative influence on the redox potential. It will become apparent, however, that only a comparatively small number of studies of this kind, applied to neoplasia, have been performed, the results of which can accordingly be regarded merely as a starting point for continued work. Furthermore, it must be stressed that biologic oxidation and reduction represent processes which depend, principally, on the presence of certain enzymes specifically adapted to the tested substrates. Because of this complex form of action, discrepancies of the results of such investigations may easily be anticipated and understood as being due to divergencies in the experimental conditions chosen. The latter will, moreover, be of essential importance for the decision, as to whether it is permissible to apply the results obtained to an interpretation of metabolic processes which occur in the living cell.

An indirect approach for investigating the oxidation-reduction systems in biologic substrates is represented by Thunberg's method, in which the reversible reduction of methylene blue into the colorless leucomethylene blue is used as an indicator.[206] This test was applied to cancerous tissues by several workers but the results obtained failed to disclose characteristic and uniform qualities (cf. chapter VI). Some authors observed a retardation of the reduction of methylene blue by malignant tumors,[207-209] whereas others found no

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206. T. L. Thunberg: Scandinav. Arch. f. Physiol. *40*: 1 (1920).
207. A. H. Drew: Brit. J. Exper. Path. *1*: 115 (1920).
208. H. Heinlein: Ztschr. f. Krebsforsch. *30*: 506 (1930).
209. A. F. Ladeck: Wien. klin. Wchnschr. *52*: 1589 (1933).

characteristic deviation from normal tissues,[210] or found an impaired reduction only in virus tumors.[211] Likewise, muscles of tumor-bearing animals did not seem to be affected regarding their behavior in the methylene blue test.[212] In an older study, it was claimed that extracts of carcinomas showed decreased ability to reduce sodium hypobromite, if compared with extracts of normal organs,[213] but a more recent investigation indicated that exudates of cancer patients contained an increased amount of substances capable of reducing ferric chloride or ferricyanide, and it was assumed that sulfhydryl compounds were primarily involved in this phenomenon.[214]

Much more valuable information has been furnished by direct electrometric measurements of the oxidation-reduction potential in neoplasms, but only few studies of this kind have been recorded so far. The aerobic oxidation-reduction potential of Jensen rat sarcoma and of spontaneous rat tumors has been found to be lower than the values of normal organs,[215] but other authors reported that colorimetric and electrometric determinations of the oxidation-reduction potential in transplanted mouse and rat tumors did not yield values differing from those of normal organs, whereas the spleen of tumor-bearing animals exhibited a markedly negative potential.[216] Another study, however, confirmed the earlier results and apparently clarified the discrepancy mentioned, inasmuch as the growing zone of animal tumors was found to possess a strongly negative potential, whereas in the necrotic tumor parts highly positive potentials were encountered. The following values may illustrate the relations observed: rat liver—plus 21 millivolt, growing zone of rat carcinoma—minus 3 millivolt, necrosis of rat carcinoma—plus 270 millivolt; rat fetus—minus 11 millivolt; growing zone of rabbit sarcoma—minus 2 millivolt, necrosis of rabbit sarcoma—plus 153 millivolt.[217] It is of interest to note the similar relations in embryonic tissue and in growing tumor parts, a finding which has also been obtained in analogous studies on Jensen rat sarcomas by comparing the oxidation-reduction potentials of the tumor with adult rat muscle and with rat embryo; the lower values observed in the tumor and in the embryonic tissue were thought to be attributable to a type of metabolism common to both tissues.[218] While a close connection between growth processes and oxidation-reduction potential has thus been suggested, the assumption of a correlation between this physico-chemical quality and the cholesterol content of tissues [219] is of a more hypothetical character.

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[210] C. Voegtlin, J. M. Johnson & H. A. Dyer: J. Pharmacol. & Exper. Ther. *24*: 305 (1924).

[211] E. S. G. Barron: J. Exper. Med. *55*: 829 (1932).

[212] B. Purjesz, L. Berkesy & K. Gonczi: Ztschr. f. Krebsforsch. *41*: 358 (1933).

[213] O. Marchetti & E. Filippi: Speriment. *57*: 181 (1903), abstr. Ztschr. f. Krebsforsch. *1*: 138 (1904).

[214] K. Apprich & F. Urban: Biochem. Ztschr. *292*: 360 (1937).

[215] E. Soru: Compt. rend. Soc. de biol. *106*: 415 (1931).

[216] R. Bierich & A. Lang: Biochem. Ztschr. *287*: 411 (1936).

This leads to attempts aimed at a chemical definition of the reducing substances, the accumulation of which might be responsible for the lowering of the oxidation-reduction potential in neoplasms. As mentioned above,[211] sulfhydryl compounds were believed to belong in this category, and in the second chapter reference has been made to the claim that a prevalence of the reduced form of glutathione over the oxidized form exists,[220] but it was stressed that the validity of this conception had been shaken by results of later experimental work. A far greater significance, however, must be attributed in this respect to ascorbic acid, viz., vitamin C, one of the most powerful reducing agents occurring in the living organism. As will be enlarged on in chapter V, an increased content of this substance has been ascertained by the analysis of malignant tumors.[221, 222] It is of great significance that this augmentation was found to be confined to the intact tumor parts, while necrotic tumor parts exhibited much lower values of ascorbic acid,[223, 224] a finding which seems to agree with the difference recorded by direct determinations of the oxidation-reduction potentials in necrotic and non-necrotic tumor parts.[217] The assumption, however, that titrametric determinations of reducing substances by means of dichlorophenol indophenol, in addition to ascorbic acid, quantitatively determined some unknown substance ("reducytin") in malignant tumors [225] cannot be accepted, since several studies arrived at identical results of the ascorbic acid content of tumors, regardless of whether titrametric or biologic assays were employed (cf. Chapter V). An accumulation of reducing substances has also been indicated by the observation that the reduced form of the cozymase is strongly prevalent in Jensen rat sarcoma, even to a higher degree than in embryonic tissue.[226] Hence, evidence derived from work undertaken from various points of view appears clearly to point to an increased presence of reducing systems in malignant tumors—a phenomenon, of which the decisive significance in metabolic processes will be discussed in chapter VI.

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[217] T. Ishikawa & K. Takigawa: Tr. Soc. path. jap. *25*: 246 (1935).

[218] H. O. Lambret, J. Swynghedauw & J. Driessens: Bull. Assoc. franç. p. l'étude du cancer *28*: 546 (1939).

[219] A. H. Roffo & E. H. Ducloux: Bol. inst. de med. exper. para el estud. y trat. d. cancer *10*: 488 (1933); 1st Internat. Cancer Congress, Madrid, *2*: 331 (1933).

[220] R. Bierich & K. Kalle: Ztschr. f. physiol. Chem. *159*: 1 (1926).

Only few data are available as to the oxidation-reduction potential of serum of cancer patients. It has been reported

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221. E. Boyland: Biochem. J. *27*: 802 (1933).
222. A. F. Watson & M. Mitolo: Biochem. J. *28*: 811 (1934).
223. S. Edlbacher & F. Koller: Ztschr. f. physiol. Chem. *227*: 99 (1934).
224. S. Edlbacher & A. Jung: Ztschr. f. physiol. Chem. *227*: 114 (1934).
225. L. J. Harris: Nature, London *132*: *27*; 605 (1933).
226. H. v. Euler, M. Malmberg & G. Günther: Ztschr. f. Krebsforsch. *45*: 425 (1937).

that the values found were generally higher than in the serum of cancer-free persons, and that they showed a marked tendency to variation, following roentgen radiation.[227] The claim of a characteristic difference, exhibited by the oxidation-reduction potentials of hexane extracts of sera of cancer patients and of cancer-free persons, respectively,[228] could not be confirmed.[229] Likewise, a statement which inferred an increased reducing power of the sera of cancer patients for methylene blue in the presence of tumor extracts[230] has been subject to contradiction.[231] A recent investigation dealt with the oxidation-reduction potentials in the blood of mice painted with benzpyrene. Normal values were determined until the actual tumor induction, then slightly lower values appeared, which decreased further with progressing tumor growth.[231a] For the sake of completeness, it may also be mentioned that an increased reducing capacity has been claimed for the urines of cancer patients,[232] although it hardly can be believed that any specific relations participate in this behavior.

As far as a possible influence of reducing substances on the cancerous process is concerned, reference may be made to a communication, according to which peroral administration of small amounts of reducing chemicals, such as sodium thiosulfate, bisulfite or hyposulfite, to cancer patients somewhat inhibited the tumor growth, and from this observation it was implied that processes of reduction may be impaired in the intestinal tract of cancer patients.[233]

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227. N. Waterman: Ztschr. f. Krebsforsch. *38* 301 (1932); Acta Cancrol. *1*: 5 (1934).

228. F. Danmeyer, J. Schubert & L. Treplin · IIe congrès internat. de la lumière, Copenhagen, 1932.

229. E. R. Holiday & F. C. Smith · Am J. Cancer *23*. 339 (1935).

230. J. Thomas & Binetti: Compt. rend. Soc. de biol. *86*: 29 (1922).

231. A. Rémond & M. Sendrail: Gaz. d. hôp. civ et mil. *97*: 1173 (1924).

231a. L. Th. Larionow: Cancer Research *1*: 860 (1941).

232. A. S. Leyton & H G. Leyton: Brit M. J. *1*: 852 (1921).

233. H. Auler, in C. Adam & H. Auler. Neuere Ergebnisse auf dem Gebiete der Krebsforschung, Leipzig, 1937, S. Hirzel, p. 303.

Concluding the discussion of this problem, the elaboration of which obviously is still in the initial stage, the great desirability of extending studies on the oxidation-reduction potential to chemically induced animal tumors may be emphasized, from which experiments information may be expected as to the phase of tumor development in which changes of this physico-chemical quality become apparent. In the scope of this work determinations of the oxidation-reduction potentials of tumors before, and after, the application of therapeutic measures should also be included, so as to ascertain whether the values registered indicate the effectiveness of a special treatment

5. *Colloid Chemistry*

The science of colloids has been supposed to contain clues for a great many biologic problems, and in fact, though still in the stage of development, has provided essential contributions which are valuable for the comprehension of phenomena connected with living matter. In this respect, the connection between hydration and *dehydration*, on the one hand, and between growth and aging, on the other, may be recalled, as well as the application of colloid chemical methods and theories to enzymatic and immunological problems. In view of these facts, it is surprising that a colloid chemical elaboration of the cancer problem has up to now been undertaken only in a very restricted manner and, in this connection, it is illustrative that modern text books on colloid chemistry in biology and medicine[234] [235] have devoted a comparatively small place to the tumor problem. Therefore, many of the experimental data, enumerated in the following, represent merely isolated, incoherent observations, frequently due to coincidental factors, rather than to a systematic colloid chemical analysis of neoplasia.

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[234] J. H. Bechhold: Die Kolloide in Biologie und Medizin, Dresden, Steinkopf, 1929.

[235] J. Alexander: Colloid Chemistry, Principles and Applications, New York, Van Nostrand Co., 1937.

Size of tumor colloids. The size of colloids is the basic property by which they are defined and differentiated from crystalloids, on the one hand, and suspensions, on the other. As far as tumor colloids are concerned, it will be recollected that in the second chapter the pertinent relations of proteins in neoplasms have been detailed. A shift of the albumin globulin ratio to the phase of lower molecular size (albumin) has been observed, and the same tendency was apparent within the globulin fraction, namely a predominance of the pseudoglobulin over the euglobulin. In addition, it has been reported that the albumin molecule in tumors was found to be of a smaller size than the molecule of normal serum albumin, and it was assumed that the rapid growth prevents the formation of a higher polymerization.[236, 237]

Colloid chemistry of cancer serum. In conjunction with this problem, also, reference is made to the findings discussed in the second chapter, that is, to the shift of the serum proteins to the fractions with higher molecular weight (globulins) which was noted as taking place in the majority of cancer patients, although this was recognized as being not regularly associated with neoplasia. Direct physico-chemical methods have been applied in ultra-microscopic studies leading to the result that the granules observed in sera of cancer patients were larger, less numerous, and less motile than those seen in sera of cancer-free persons.[180] Similarly, changes of the corpuscles visible in dark field examination of sera have been reported when the tested substrates were derived from patients with malignancy, but this occurred also in sera of persons with proliferative processes; especially after the addition of acid, a much larger size of the particles

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[236] A. Boutaric: Protoplasma *18*: 294 (1933).

[237] C. Achard & M. Piettre: Compt. rend. Acad. d. sc. *201*: 751 (1935).

than in normal sera was seen in sera of cancer patients.[238] Following administration of radium-emanation, in vivo or in vitro, the colloid structure of cancer serum was found to approach the normal state,[130] thus demonstrating a therapeutic measure which influenced physicochemical qualities. Roentgenographic investigation of the molecular structure of cancer, and of normal sera, showed certain deviations regarding details of the pictures obtained, but the establishment of definitely characteristic criteria was not feasible.[239] After mixture of exudates of cancer patients with sera of varying origin, changes of the colloidal structure of the substrates have been detected by means of nephelometric and photometric methods,[240] but an interpretation of these complex relations meets with difficulties. In general, it may be stated that a disturbance of the colloidal structure, viz., a decrease of the degree of dispersion, seems to prevail in the sera of cancer patients, without it being possible to consider these phenomena as being primarily due to neoplasia. As will be shown in chapter X, the increased colloidal "lability" of sera of tumor patients has formed the basis for a number of diagnostic tests, none of which, however, has proved to be specific to any extent for malignant diseases.

Studies of the behavior of sera, regarding their activity as protective colloids, are closely connected with the findings discussed above. In satisfactory agreement with the observation of an increased size of the colloidal particles in cancer serum, it has been reported that a great majority of these sera also exhibited an impaired ability to act as protective colloids. Such results have been obtained by using Congo rubine in eighty per cent of the cancer sera tested,[241] or in seventy-five per cent, using suspensions of Prussian blue,[242] and alterations have been observed in the gold sol precipitation when ultrafiltrates of cancer sera were employed, although no sharp delineation of this behavior against sera of cancer-free persons was possible.[243] The majority of cancer sera were also found to cause precipitation of aqueous lecithin emulsions,[244] and, as has been clearly shown, this phenomenon is by no means connected with immunological reactions, but it is solely an expression of the disturbed colloidal serum structure,[245] and, therefore, related to the so-called lability reactions

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[238] W. Fischer: Arch. f. klin. Chir. *185*: 189 (1936).
[239] H. Yamakawa & T. Nakamura: Gann *30*: 22 (1936).
[240] M. Berliner: Ztschr. f. Krebsforsch. *32*: 171 (1931).
[241] L. F. Loeb: Ztschr. f. Krebsforsch. *20*: 432 (1923).

Several studies dealt directly with the colloidal qualities of serum proteins in neoplasia, thus supplementing the chemical investigations discussed in chapter II. The coagulation point of proteins has been found raised in sera of cancer patients, but also of patients suffering from diabetes, icterus, or pernicious anemia,[246] and ten times diluted sera of tumor patients, heated to 80° C, exhibited a weaker opalescence than normal sera treated in the same way [247] The time necessary for the coagulation of serum at 65 to 75° C was found markedly lengthened when the specimens were derived from cancer patients or from rats with Jensen sarcoma; in the latter case, the degree of this change ran parallel to the tumor-size of the animals.[248] In recent studies, it was observed that the heat coagulation of sera may proceed in two clearly distinguishable ways. Type 1 comprises those cases in which,

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242. E. L. Lederer: Strahlentherapie *42*. 675 (1931).
243. F. Chrometzka: Deutsche med Wchnschr. *59*: 1288 (1933); Klin. Wchnschr. *15* · 580 (1936).
244. F. Weil & H. Braun: Wien. klin. Wchnschr. *21*: 938 (1908).
245. H. Sachs: Fragen der Krebsforschung; Beihefte zur Med. Klin. 1931, H. 2, pp. 87 seq.
246. R. L. Mayer: Klin. Wchnschr. *1*. 1693 (1922); G. Rosenow: Klin. Wchnschr. *1*: 1694 (1922).
247. O. Ehrenteil & W. Weis-Ostborn: Klin Wchnschr. *2* · 585 (1923).
248. B. Purjesz: Ztschr. f. Krebsforsch. *44*: 1 (1936).

after cooling and reheating of the samples, the coagulated protein adheres to the wall of the test tube, releasing an opalescent fatty salt solution, whereas in type II the serum is jellified as a whole, and remains unchanged by repeated heating. The majority of sera of cancer patients was found to belong to type II, although sera showed an analogous behavior also in some other pathologies, especially so in pneumonia.[249] Re-examinations of this phenomenon confirmed the original findings, but stressed its unspecific nature.[250, 251] As far as an interpretation of these deviations observed for the coagulation of cancer sera is concerned, it must be borne in mind that they are not necessarily dependent solely on changes of the colloidal protein structure, but are also influenced by other factors, especially by the hypoproteinemia, as well as by alterations of the electrolyte content of the serum. An accelerated gel formation has been claimed to result from the addition of lactic acid to cancer serum,[252] but this effect has been recognized as being unspecific, since it was also encountered in sera of pregnancy,[253] or in luetics and lepers.[254] A method for clinical investigation of the colloidal serum structure has been devised, which consists in determining the coagulation of diluted serum in the presence of varying calcium chloride concentrations (Weltmann's coagulation band),[255] a test which has been found useful for the evaluation of certain pathologies, especially of pulmonary tuberculosis. Numerous experiments in which this test was applied to neoplastic diseases agreed in that in the majority

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249. H. F. Kurten: Klin. Wchnschr *18*: 667 (1939).
250. O. v. Soden & K. Dirr: Ztschr. f. d. ges. exper Med. *108*: 159 (1940).
251. W. Zumke: Klin. Wchnschr. *20*. 53 (1941).
252 W. Kopaczewski: Ztschr. f. Krebsforsch. *42*: 262 (1935).
253. G. Legrand: Gynécologie *34*: 239 (1935).
254. R. Gillier: Compt. rend. Soc. de biol. *118*: 1324 (1935).
255. O. Weltmann: Wien. klin. Wchnschr. *43*: 1304 (1930).

of sera of cancer patients a "shortening" of the coagulation band was apparent, that is, coagulation was observed only in the higher calcium chloride concentrations, but it was declared that this finding was by no means characteristic for malignant tumors, and was probably dependent on secondary factors, such as tumor necrosis.[256-262] Likewise, another coagulation test, using a dilution series of sera under addition of magnesium chloride,[263] failed to reveal characteristic relations when applied to the sera of tumor patients,[264] and an investigation employing a combined physicochemical analysis of sera, such as flocculation under specific conditions, refractory index, etc., yielded results of too uncertain nature as to form a basis for a successful differentiation.[265]

Hydration. As has been elaborated in chapter I, clear cut evidence has been furnished for the presence of an increased water content in neoplasms, and the factors thought responsible for this phenomenon, as well as its possible significance, have been discussed and more especially the connection with growth processes has been stressed, as demonstrated by similar relations in embryonic tissue. At this point, however, not simply the water content is of interest, but principally the fraction bound intramolecularly as hydration water. An elucidation of this problem was attempted in the following manner. In an extensive study, neoplastic and normal tissues were subjected to drying at varying

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256. H. Ypsilanti & L. Kyriakis: Zentralbl. f. Gynäk. *12*: 22 (1934).
257. G. Carriere, P. Martin & J. Driessens: Compt. rend. Soc. de biol. *120*: 31 (1935).
258. O. Roeloffs: Klin. Wchnschr. *15*: 1802 (1936).
259. R. Teufl: Wien. Arch. f. inn. Med. *29*: 297 (1936).
260. U. Borghetti: Tumori n.s. *10*: 187 (1936).
261. H. Rosegger: Ztschr. f. klin. Med. *134*: 495 (1938).
262. A. B. Cairns & L. Brandenstein: Texas State J. Med. *36*: 22 (1940).
263. R. Bauer: Med. Klin. *30*: 230 (1934).
264. J. Kretz: Wien. klin. Wchnschr. *46*: 492 (1933).
265. R. Jonnard: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *14*: 141 (1937).

temperatures, and the consecutive water loss was determined. The curves plotted from these values exhibited characteristic breaks, which were considered by the authors as indicating the demarcation of "free" and "bound" water content of the tissues. Although neoplastic tissues did not show definite deviations as to the absolute values, the comparison of the curves revealed marked differences as to the kinetics of the water loss during desiccation. In this respect, it seemed especially significant that the reversibility of desiccation was definitely impaired in tumor tissue. After complete deprivation of water, tumor tissue was capable of taking up again a maximum of only nine per cent of the original water content, whereas the degree of reversibility reached eighteen per cent in normal tissues.[266] Hence neoplastic tissue appears to possess a greater tendency than normal tissue to hysteresis, i.e., aging in the colloid chemical sense. These observations agree well with an older report, according to which neoplastic tissues are much more affected by loss of water than normal or embryonic cells,[267] although the latter contain approximately the same amount of water as cancer cells; one will hardly go amiss in assuming that the demonstrated irreversibility of desiccation in tumor cells is responsible for this increased sensitivity against water deprivation, and a similar explanation may be applied to the finding that rat sarcomas lost their transplantability after being dried over calcium chloride for twenty-four hours.[268]

Experiments on the swelling of neoplastic cells in vitro have yielded less informative results. One author found no difference in the water intake between normal and tumor tissue, but he stated that a previous treatment with calcium chloride caused an increased swelling of normal cells, whereas tumor cells reacted in the opposite manner.[269] The resistance of tumor cells against the dehydrating effect of hypertonic solutions was claimed to be less than that of normal cells, as demonstrated by observation of tissue cultures and by reimplantation; carcinomatous cells were found more sensitive in this respect than sarcomatous ones.[270] However, other investigations performed with very exact methods failed to demonstrate definite quantitative deviations from the normal of the swelling of neoplastic cells in vitro,[271, 272] and accurate measurements of volume and surface did not reveal a different behavior of normal and of sarcomatous fibroblasts when subjected, in tissue cultures, to the action of hypotonic solutions.[273] Continued studies of osmotic influences on mouse sarcoma cells showed that protein-free salt solutions caused an increase of the swelling, parallel to the salt concentration, whereas the addition of protein, to some extent, inhibited the swelling. This swelling of the cells did not affect their vitality, as was experimentally proven[274] In the evaluation of these experiments, it must be remembered that the phenomena observed depend only in part on the hydrophilia of the cell colloids, but are also decisively determined by the permeability of the cancer cell membranes

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[266] N. Okuneff & T. Nasarbekowa: Ztschr. f. Krebsforsch. *41*: 28 (1934).

[267] A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat. d cáncer *7*: 596 (1930).

[268] S. Morimoto: Acta dermat. *22*: 48 (1933); abstr. Am. J. Cancer *22*: 414 (1934).

Osmotic pressure has been found increased in Flexner-Jobling rat carcinoma cells from three to four times the normal value, in Jensen sarcoma cells from six to seven times the normal value[275] The hydremia, frequently observed in the serum of cancer patients, obviously is a secondary consequence of cachexia and hypoproteinemia, and likewise a diminution of the oncotic pressure of the serum proteins, found by some authors in cancer patients,[152, 276, 277] is of unspecific nature, since it is caused by a prevalence of globulin over the albumin, which latter occurs in a state of higher hydration. But certain qualitative deviations of the hydration of serum colloids have been made probable by an observation that the configurations visible after desiccation of serum drops showed a different form when the specimen was derived from a cancer patient.[278] Similarly, the crystallization of sodium chloride, added to fowl serum, has been reported to yield a pattern deviating from the normal, when the animals tested had been inoculated with sarcoma;[279, 280] sera of cancer patients, however, did not seem to influence the crystallization of sodium chloride.[281] In the same category a report may be classified, according to which different crystal patterns developed when mixtures of nickel chloride and potassium sulfate were added to sera of normal, or of tumor-bearing rabbits, respectively, and these findings were considered as indicating a change of the micellar structure in the serum of tumor animals.[282] A greater amount of work has been done relating to the crystal patterns of mixtures of serum with copper sulfate, the resulting structures were claimed to be specifically influenced by certain pathologic conditions, among which neoplasia should lead to a characteristic pattern, distinguishable, both from the behavior of normal sera, or from

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[269] M. A Magath: Ztschr. f Krebsforsch. *24*: 126 (1926).

[270] H. Auler Ztschr. f. Krebsforsch *44*: 332 (1936).

[271] M. J. Shear & L. C Fogg. Pub Health Rep. *49*: 225 (1934).

[272] M. J Shear: Am. J. Cancer *23*: 771 (1935).

[273] A. M. Brues & C. McTiernan Master: Am. J. Cancer *28*: 314 (1936).

[274] M. Belkin & M. J. Shear: Am. J Cancer *32*: 62 (1938).

[275] H. Auler: Libro de Oro, Buenos Aires, p. 56 (1935).

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276. H. Guthmann & H. Fruhauf: Arch f. Gynak *31*: 431 (1928).
277. S. Marino & D. Trapani: Riforma med *52*: 423 (1936), abstr. Ztschr. f. Krebsforsch. *44*: Ref. *232* (1936).
278. R. Douris & C. Mondain: Compt. rend Acad. d. sc *185*: 232 (1927).
279. F. Herčik: Ztschr f. Krebsforsch. *33*: 146 (1930).
280. V. Kilian: Acta Cancrol. *1*: 22 (1934).
281. M. Fořt & V. Morávek: Acta Cancrol. *2*: 51 (1936).
282. T. Kofman: Compt. rend. Acad. d. sc. *206*: 75 (1938).

those derived from patients suffering from other diseases.[283] A diagnostic application of this phenomenon has been proposed, and a number of investigations actually achieved a far reaching agreement between the clinical diagnosis and the results obtained with the test mentioned.[284-286]

Determinations of the isoelectric point. It has been established that the hydration of protein colloids is closely connected with their electric charge, inasmuch as every change of this state is accompanied, primarily, by an increased tendency to molecular water intake, and that the hydration of proteins is smallest at the hydrogen ion concentration which entails the lowest degree of protein ionization; the corresponding value of the pH is designated as the isoelectric point (pHi).[287] For this reason, studies of the isoelectric point of cancerous tissues may be of interest and actually were performed by a number of workers.[288] It was observed that the pHi in the muscles of tumor-bearing mice showed an increase, i.e., a shift to the alkaline side, soon after the tumor inoculation, and analogous findings were reported for tar cancer and spontaneous tumors of mice. These changes, however, cannot be regarded as being specific for neoplasia, since similar alterations occurred during infections of the animals, or they could be produced experimentally by the following means. Injection of turpentine caused a shift of the pHi to the alkaline side and injection of zinc, gold, iron or coal suspensions a shift to the acid side. But it is noteworthy that these

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283. E. Pfeiffer: Empfindliche Kristallisationsvorgänge als Nachweis von Formungskräften im Blute, Dresden, Verl. E. Weise, 1935; 3rd Internat. Cancer Congress, Atlantic City, p. 198 (1939).

284. M. P. Bégouin: Bull. acad. de méd., Paris *119*: 746 (1938).

285. S. Rascher & J. Trumpp: München. med. Wchnschr. *86*: 544 (1939).

286. O. C. Gruner: Canada. M. A. J. *43*: 99 (1940).

287. W. Pauli: Eiweisskörper und Kolloide, Wien, Springer, 1926.

288. F. Vlès & A. de Coulon: Strasbourg Méd. *85*: 1 (1927); 2nd Internat. Cancer Congress, Madrid, *2*: 379 (1933).

experimentally induced changes of the isoelectric point appeared to affect the development of subsequently inoculated tumors in a specific way, inasmuch as interferences, accompanied by a more alkaline pHi, enhanced tumor growth, whereas the opposite effect followed a shift of the pHi to the acid side. On the basis of these observations, and, furthermore, because a more alkaline pHi of the serum proteins was also found in cancer patients, the authors viewed this change of the isoelectric point as a positive factor for predisposition to neoplasia. Later work confirmed the fact that the pHi is shifted to the alkaline side in serum of cancer patients (pHi of normal male—5.5; of normal female—6.0; of cancer patients—over 7.0), but similar deviations were also found in other diseases.[289] As to malignant tumors themselves, the isoelectric point has been determined in Ehrlich mouse carcinoma and in human neoplasms by an indirect method based on the principle that the pHi coincides with that pH which causes dyes to produce the weakest staining effect in cells [290] and, in this way, the isoelectric point of the cellular structure of tumors was found to be more acid and to be subject to greater variations than the corresponding relations in normal tissues.[291] It is regrettable, in view of these apparently divergent claims—more alkaline pHi in the tumor-affected organism,[288] and more acid pHi in the tumors [291]—that no investigations employed the same method for a determination of both values. One remark may be added to the circumstance of the experimental changes of the isoelectric point, as mentioned above.[288] It seems of interest that the colloids used for this purpose are such as are stored by the reticulo-endothelial system, and hence they may influence its functional activity. Furthermore, the ability of

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289. M. Faguet: Bull. Assoc. franç. p. l'ètude du cancer *25*: 645 (1936).
290. A. Pischinger: Ztschr. f. Zellforsch. u. mikr. Anat. *5*: 347 (1927).
291. M. Solowiewa: Acta Cancrol. *2*: 321 (1936).

regulating the degree of dispersion of biocolloids has been ascribed to the reticulo-endothelial apparatus,[292] wherefore it appears possible that this system may also determine the electrical charge and the isoelectric point, respectively, of colloids in the body, and a new aspect is disclosed in regard to the correlation between reticulo-endothelial system and neoplasia, a subject discussed in detail in chapter VIII.

Miscellaneous physicochemical qualities. A decreased electrical conductivity, found in about seventy-five per cent of the tested sera of cancer patients,[293, 294] most likely is due to quantitative and qualitative changes of the electrolyte content, and is therefore obviously of a nonspecific nature. Likewise, the depression of the freezing point is a physico-chemical quality dependent on variations of the inorganic constitution, since the freezing point of a medium decreases proportionally to the amounts of salts, or ions, contained in it. The lowering of the freezing point, determined in animal tumors, corresponded approximately to the values of normal organs,[295] and normal ($\Delta = 0.56$ to 0.58) or slightly diminished values (0.53) have been observed in the sera of cancer patients.[296] The slight decrease may be due to hydremia, while older statements inferring a stronger lowering of the freezing point in some cancer sera [297, 298] have been explained as being caused by secondary complications, such as compression of the ureters by the tumors and consequent sodium chloride retention in the blood. On the other hand, an increased viscosity, observed by several authors in the sera of cancer patients,[135, 276, 293] may be a consequence of the aforementioned alteration of the colloids, that is, of an increase of the size of the particles in these sera. By means of an indirect approach, it was believed possible to demonstrate the existence of an increased viscosity of tumor cells since, after being subjected to centrifuging, their cellular content did not show a displacement or did so only to a much smaller extent than normal cells under the same condition. In this manner, various transplanted and spontaneous mouse tumors were studied; cancerous cells seemed to exhibit a greater resistance against displacement of their contents by centrifugal force than that shown by sarcomatous cells, and this stiffening of the cellular structure, it was assumed, might account for the mitotic anomalies encountered in neoplastic cells.[299] In continuation of this work, an increased viscosity was also claimed for the cells of rat hepatomas induced by butter yellow. In contrast to normal liver cells, the hepatoma cells were found unstratified after being subjected to high-speed centrifuging. This alteration was noticeable even at an early stage of tumor formation and was tentatively assumed to be caused by clotting of the proteins by lactic acid.[300] Other investigators, however, raised objections against the experiments mentioned, and their observations pointed to a decrease of the viscosity in the nuclei of neoplastic cells. Hence they were not in complete contradiction with the first named findings, which related mainly to the cytoplasma. In the later investigation, the nuclear viscosity of normal mouse skin cells, measured by their centrifugal displaceability, was compared with the quality of skin of animals treated with methylcholanthrene, and a decrease was found to accompany the papilloma formation, whereas the lowest values were obtained in the cells of the tumors finally arising. Similar relations appeared to prevail in human skin, since normal cells exhibited higher values of nuclear viscosity than cells of healing wounds, or of papillomas, and the lowest values again occurred in squamous cell carcinomas.[301]

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[292] S. Bratiano & A. Llombart Compt rend. Soc. de biol. *101*: 299 (1929).

[293] de Georgi; quot. J. M G. Galvan: 1st Internat. Cancer Congress, Madrid, *2*: 528 (1933)

[294] F. Steigerwaldt & B Reiser Arch. exper. Path. & Pharmakol. *173*: 15 (1933).

[295] E. Howard: Am. J. Cancer *23* · 87 (1935).

[296] K. Engel: Berl. klin. Wchnschr *41* 828 (1904).

[297] J. Israel: Mitt. a d. Grenzgeb d. Med u Chir. *11*: 171 (1903).

[298] F. Engelmann: Mitt. a.d. Grenzgeb. d Med. u. Chir. *12*: 396 (1903).

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[299] M. F. Guyer & P. E. Claus: Proc. Soc. Exper. Biol. & Med. *35*: 468 (1935); Anat. Rec. *73*: 17 (1939).

[300] M. F. Guyer & P. E. Claus: Cancer Research *2*: 16 (1942).

A very complex phenomenon is represented by the sedimentation rate of erythrocytes, the mechanism of which has not yet been fully clarified, in spite of its extensive clinical application. It has, however, been recognized that the outcome of this test is determined by a great many chemical and physicochemical properties, both of the red blood corpuscles (size, electric charge) and of the plasma (content of fibrinogen, albumin and globulin, water content, viscosity, surface tension, etc.). Because of the simple technical procedure, so many clinical studies on the sedimentation rate in neoplasia have been published that it is not possible here to mention more than a small part of them.[302-318] Most of the authors agreed on the following points: advanced malignant tumors almost regularly show an increased sedimentation rate, probably due to tumor necrosis, while in early stages normal values may very frequently be observed. Although

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301. E. V. Cowdry & F. X. Paletta: Am. J. Path. *17*: 335 (1941).
302. W. Sauer: Biochem. Ztschr. *168*: 335 (1926).
303. P. Goldschmidt-Fürstner: Ztschr. f. Krebsforsch. *30*: 241 (1930).
304. E. Berthold: Monatschr. f. Krebsbekämpf. *1*: 449 (1933).
305. Best, Fromme, Payr et al: Anleitung zur frühzeitigen Erkennung der Krebskrankheit, Leipzig, 1932.
306. H. Reichel: Blutkörperchensenkung, Vienna, Springer, 1936.
307. H. Guthmann & G. H. Schneider: Arch. f. Gynäk. *127*: 515 (1926).
308. A. Giesecke: Zentralbl. f. Gynäk. *45*: 369 (1921).
309. R. Maislisch: Ztschr. f. Krebsforsch. *31*: 605 (1930).
310. F. K. Kessel: Arch. f. klin. Chir. *151*: 811 (1928).
311. F. Lickint: Med. Klin. *24*: 1314 (1928).
312. J. Adams-Ray: Acta chir. Scandinav. *66*: 263 (1930).
313. R. Nicole: Bull. Schweiz. Ver. gg. Krebsbek. *2*: 76 (1935).

accelerated sedimentation rates are associated with a great number of different pathologies, nevertheless, such a finding in clinically unclear cases should cause a suspicion of the existence of neoplasia, and the test may be useful for the differentiation of benign and of malignant tumors,[314] or for the detection of metastases formation.[315] Attempts have been made by various means to modify the technique of the sedimentation rate by creating experimental conditions which might lead to more characteristic results in neoplasia. The addition of various chemicals to the blood, or of substances isolated from tumors, did not yield findings of practical value.[316] It has been reported that red blood cells of cancer patients, transferred into normal or cancerous serum which had previously acted on cancer cells in vitro and had been separated from them, showed a slower sedimentation rate than in the original plasma, whereas normal erythrocytes did not react in this manner.[317] However, no further studies on this effect have been recorded nor has substantiation been given to another claim of the different behavior of normal and of cancerous blood—the occurrence of a gradual slowing of the sedimentation, when the test was repeated during the twenty-four hours after the withdrawal of normal blood, but not of blood of cancer patients;[12] a re-examination failed to confirm this phenomenon.[318] In addition, another sedimentation test, in which a sodium hydroxide solution was stratified over the coagulated serum and the velocity of the diffusion of the base into the serum was determined, has been devised, and an accelerated diffusion was claimed to prevail in cancer sera.[319] A later study, however, demonstrated this finding to be unspecific, because it depended on a protein diminution of the serum.[320]

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[314] J. M. Feder: J. South Carolina M. A. *35*: 102 (1939).

[315] S. A. Wolfson, S. Reznik & L. Gunther: J. A. M. A. *116*: 1044 (1941).

[316] S. Sterling-Okuniewski & W. Grundgand: 1st Internat. Cancer Congress, Madrid, *2*: 609 (1933).

[317] G. H. Smith & F. Mack: Yale J. Biol. & Med. *9*: 173 (1936).

[318] F. Sanides: Ztschr. f. Krebsforsch. *48*: 21 (1938).

6. *Biophysics.*

Lately, a gradual tendency has been to join physical chemistry with pure physics in general. A close interdependence exists particularly in regard to the physical qualities of biologic substrates and the action of physical energies on living matter, on the one side, and their physicochemical structure, on the other side. For this reason, a supplementary and rather brief survey of biophysical data relating to neoplasia may conclude this chapter, although these problems cannot be regarded as strictly within the scope of this treatise.

Only brief reference will be made to connections between *mechanical stimuli* and tumor development, a problem which has given rise to an abundant literature (cf.[321, 322]) relating to case histories of tumor formation—mostly of sarcomas—following a mechanical trauma, and in these instances it is frequently of the greatest practical importance, on account of questions regarding insurance obligations and legal procedures, whether a causal nexus between these two events can be acknowledged. In animal experiments, precancerous alterations were reported to have resulted from repeated mechanical traumas applied to ears of rabbits and guinea pigs.[323] On the other hand, a less familiar finding may be mentioned, according to which it should be possible to induce regression of transplanted rat tumors by consist-

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319 B. Serény: Deutsches Arch. f. klin. Med. *180*: 630 (1937); Deutsche med. Wchnschr. *66*: 193 (1938).

320. K. Ko: Klin. Wchnschr *19*: 60 (1940).

321. F. Blumenthal: Ergebnisse der experimentellen Krebsforschung, Leiden, 1935.

322. J. J. M. Shaw: Lancet *1*: 860 (1935).

323. F. D. Rezzesi: abstr. Ztschr. f. Krebsforsch. *44*: Ref. 210 (1936).

ently repeated mechanical damage in the form of massage and application of pressure to the tumors.[324] However, it appears very likely that the tumor strain used in these experiments possessed an inherent tendency to spontaneous regression, which was enhanced by the vascular damage caused by the mechanical traumas.

Electricity. A few measurements of the electrical charge have been performed in neoplastic material, and it was claimed that a disturbance of the electrical balance existed in tumor cells, resulting in an electric potential from the stronger positive cell nucleus to the less charged cytoplasma.[325] Furthermore, it was reported that the electrical charge of tumors differed from that of the surrounding normal tissue,[326] and several workers found in malignant tumors a decreased electric resistance, a decreased electric polarization, and an increased electric conductivity.[3, 325-328] The importance of these findings with relation to the tumor cell permeability has been discussed in the first section of this chapter, and it would also seem possible to connect the changes of these physical properties with the increased water content of neoplasms. Much more significant phenomema have been revealed in extensive studies, performed within the last years, on the bioelectric fields of animals, in connection with normal growth and tumor development.[329-331] These measurements demonstrated a characteristic difference between strains of mice with different genetic qualities as to the incidence of spontaneous tumors; the formation of tumors, as well as the process of normal aging, was seen to result in specific changes of the electrodynamic fields of the body. Similar changes were observed, in this respect, regardless of whether the tumors were spontaneous, inoculated, or chemically induced by benzpyrene or methylcholanthrene, and since the bioelectric alteration was found to precede the morphologic tumor development, it was assumed that it represented a hereditary factor, predisposing a tendency to neoplasia, which disease would result from an "impact of some untoward environmental circumstance upon a defective (electrodynamic) field, passed on from one generation to the next." [331]

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[324] H. Hirschfeld: Ztschr. f. Krebsforsch. *16*: 33 (1919).

[325] D. C. A. Butts: Cancer (N. Y.) *1*: 243 (1924).

[326] G. W. Crile, M. Telkes & A. F. Rowland: Am. J. Cancer *15*: 2659 (1931).

[327] G. W. Crile: J. Cancer Res. *9*: 388 (1925).

[328] W. C. M. Lewis: Brit. M. J. *2*: 920 (1926).

[329] H. S. Burr, G. M. Smith & L. C. Strong: Am. J. Cancer *32*: 240 (1938); Yale J. Biol. & Med. *12*: 711 (1940).

[330] H. S. Burr, L. C. Strong & G. M. Smith: Yale J. Biol, & Med. *10*: 539 (1938).

[331] H. S. Burr: a) Yale J. Biol. & Med. *12*: 277 (1940); b) *13*: 783 (1941).

These relations seem to impart a new aspect to older investigations, according to which spontaneous tumors arose more frequently in mice kept in grounded cages, in contrast to those living in cages which were electrically insulated, and in which an inhibitory effect of electrical insulation for the formation of tar cancer in mice was also claimed.[332] These findings, however, could not be confirmed by other workers, who failed to notice any influence on the development of spontaneous or tar tumors from electrical charging, or insulation, of the animal cages.[333, 334] On the other hand, according to a recent communication, exposure of mice to air, which was brought to a strong negative ionization by means of a unipolar high frequency apparatus, delayed the appearance and impeded the growth of transplanted tumors, and a similar inhibitory effect of this measure was noted on spon-

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332. F. Vlès & A. de Coulon: Compt. rend. Acad. d. sc. *195*: 586 (1932); *200*: 1435 (1935); 1st Internat. Cancer Congress, Madrid, *2*: 392 (1933); Arch. physique biol. *13*: 156 (1936).

333. H. L. Van Vierssen Trip: Nederl. tijdschr. v. geneesk. *77*: 865 (1933); abstr. Ztschr. f. Krebsforsch. *39*: Ref. 85 (1933).

334. H. Beitzke: Wien. klin. Wchnschr. *48*: 959 (1935).

taneous tumors, as well as on benzpyrene tumors.[335] Direct application of high frequency waves has been tried in a few animal experiments, but no influence of this procedure was seen on Rous sarcoma,[336] whereas a growth inhibition, and sometimes regression, of mouse carcinomas, following bipolar high frequency treatment, was accompanied by an increased death rate of the animals, probably due to overheating.[337]

The *short wave* treatment, which has attained the rôle of a valuable therapeutic measure in various fields of medicine, has also been applied to neoplastic conditions, mostly in animal experiments, but the results obtained, as well as some clinical experiences, failed to furnish clear cut evidence for its effectiveness. Although it was claimed that ultra short waves caused a more severe metabolic disturbance in transplanted mouse tumors than in normal tissue, thus creating the possibility of selectively damaging neoplastic growths,[338] ultra short wave treatments resulted, in vivo, only in a temporary growth inhibition of Jensen rat sarcoma, followed by resumed growth,[339] or even stimulated the growth of Ehrlich mouse carcinoma, though a slight prolongation of the life of the animals themselves was seen.[340] Tissue cultures of carcinomatous and sarcomatous cells were reported to be more sensitive against short wave radiation than normal or fetal tissue, and subsequent re-inoculation of the treated tumor tissue indicated decreased vitality and malignancy.[341] But a great number of later investigations were not capable of demonstrating favorable effects derived from short wave application to animal tumors, or to some cancer patients,[336,

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335. E. Küstner & C. Dittmar: Ztschr. f. Krebsforsch. *50*: 457 (1940).
336. J. R. Ross: Am. J. Cancer *18*: 905 (1933).
337. F. Ludwig & J. von Ries: Ztschr. f. Krebsforsch. *40*: 117 (1933).
338. T. Reiter: Deutsche med. Wchnschr. *59*: 1497 (1933).
339. E. Pflomm: München. med. Wchnschr. *77*: 1854 (1930).
340. P. Rondoni: Atti, acad. naz. Lincei VI s. *14*: 169 (1931).
341. A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *9*: 211 (1932); *10*: 538 (1933).

[342-349] and especially claims advanced by some authors as to a specific favorable action of certain wave lengths on animal tumors[350] cannot be accepted, in view of the fact that all tumor-inhibitory effects which could be ascribed to short wave treatment have been clearly demonstrated to depend solely on their thermal action.[351-354] Therefore interpretations of these findings necessarily must take into account thermal factors, which are discussed later. Controversial opinions have been expressed regarding the advisability of a combination of short wave and roentgen or radium therapy of malignant tumors. Reports of favorable experiences gathered with such a combination treatment in animal experiments,[355, 356, 353] or in some cancer patients,[357] are opposed by other statements denying that an increased activation of the roentgen effect followed a preceding short wave administration to animal tumors,[358, 359] or to human neoplasms.[348] There is no

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342. M. Haas & A. Lob: Strahlentherapie *50*: 345 (1934).
343. A. Eidinow: Brit. M. J. *2*: 332 (1934).
344. E. Hasché & W. A. Collier: Strahlentherapie *51*: 309 (1934).
345. P. Liebesny: Kurz-und Ultrakurzwellen, Urban & Schwarzenberg, Vienna, 1935, pp. 138 seq.
346. E. Raab: Die Kurzwellen in der Medizin, Radionta Verl., Berlin, 1935.
347. K. P. Markuse, D. A. Losinsky & N. Malow: Ztschr. f. Krebsforsch. *44*: 415 (1936).
348. S. Baumeyer: Strahlentherapie *62*: 373 (1938).
349. E. Hasché: Strahlentherapie *65*: 664 (1939).
350. H. Nakamura, M. Yamada, K. Tanaka, M. Wakabayashi & S. Sasada: Gann *30*: 548 (1936).
351. F. Dickens, S. F. Evans & H. Weil-Malherbe: Am. J. Cancer *31*: 341 (1932).
352. K. Overgaard: Acta Radiol. (Stockholm) *17*: 182 (1936).
353. K. Overgaard & H. Okkels: Strahlentherapie *68*: 587 (1940).
354. S. G. Ochakovskaya: Arch. biol. nauk. *39*: 137 (1935); abstr. Ztschr. f. Krebsforsch. *44*: Ref. 192 (1936).
355. L. Hill: Brit. M. J. *2*: 370 (1934).
356. H. & M. Langendorff: Strahlentherapie *64*: 512 (1939).
357. G. Fuchs: Klin. Wchnschr. *14*: 1582 (1935).
358. A. Lob: Monatschr. f. Krebsbekämpf. *3*: 75 (1935).
359. H. J. Taylor: Brit. J. Radiol. *9*: 467 (1936).

doubt that the material tested thus far is much too small to warrant final conclusions.

Thermal energy. Here again an extensive clinical literature is devoted to the case histories of patients, in whom tumor development has been observed following the exposure of body parts to intensive and prolonged heat radiation, such as is occupationally the case, for instance, in glass blowers or blacksmiths.[cf. 321, 360, 361] A familiar example is also the so-called Kangri cancer of Tibetans, which is due to the action of a primitive heating element carried almost constantly on the body. Dealing with the carcinogenic effect of heat those instances must be separated, in which the neoplastic growth arises due to heat radiation without previous scar formation, from those instances where such an event takes place after burns or scalding, since scars of thermal origin seem to represent a predisposing factor for carcinogenesis.[362] This leads to those clinical cases where the action of heat was combined with chemical stimuli, such as scalding with hot tar. It has been demonstrated experimentally that, when burns were produced in tarred animals far from the site of the tar application, tumors developed in these artificial injuries which had not been directly exposed to the chemical agent.[363, 364]

The possibility of inducing tumors by means of the stimulus of low temperature has been demonstrated in experiments in which repeated freezing of mouse skin with dry ice (carbon

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360. K. Ullmann: Krebsbildung in der Gewerbemedizin, Hdb. f. Haut-u. Geschlechtskrankheiten, Jadassohn, 12. Vol., pt. III, pp. 551–720 (1930).

361. L. Cornil, J. E. Paillas & H. Bonneau: Bull. Assoc. franç. p. l'étude du cancer *28*: 359 (1939).

362. A. H. Roffo & A. Gandolfo: Monatschr. f. Krebsbekämpf. *2*: 289 (1934).

363. H. T. Deelman: Bull. Assoc. franç. p. l'étude du cancer *12*: 24 (1923).

364. B. Fischer-Wasels & W. Büngeler: Virchows Arch. f. path. Anat. *275*: 723 (1929).

dioxide snow) resulted in a few instances in the development of papillomas and carcinomas.[365, 366]

A great many studies have been devoted to the analysis of the resistance of neoplasms against high, or low, temperatures. Ehrlich,[367] in his early experiments on the transplantable mouse carcinoma, named after him, observed that these tumors showed increased sensitivity against high temperatures, but exhibited an increased resistance against low temperature. It is remarkable, in this respect, that he successfully inoculated tumors which had been stored at 0° C for two years. As far as the action of heat is concerned, it was reported that increasing the temperature in mouse tumors to 44° C, prior to transplantation, decreased their virulence, and that this effect was proportional to the time of the exposure to heat.[368] In agreement with this finding, several workers found an increased heat sensitivity of tumor tissue, both if their vitality was judged by means of re-implantation [369-373] or by the results of measurements of their metabolism in vitro.[374] In one study, however, an increased heat resistance of cancer cells was claimed, on the basis of determination of the phosphate diffusion in vitro,[375] but it appears doubtful whether these experiments represented an adequate criterion for the vitality of the cancer cells, since the changes of the

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365. I. Berenblum: Brit. J. Exper. Path. *11*: 208 (1930).
366. B. J. Mansens: Nederl. tijdschr. v. geneesk. *75*: 1444 (1931).
367. P. Ehrlich, quot. Apolant, Kolle & Wassermann: Handb. d. pathogenen Mikroorganismen, Fischer, Jena, 1913, Vol. 13, p. 167.
368. E. P. C. White & L. Loeb: Zentralbl. f. Bakteriol. Orig. *56*: 325 (1910).
369. C. O. Jensen: Zentralbl. f. Bakteriol. *34*: 28; 122 (1903).
370. Bashford; Hertwig & Poll; quot. C. Lewin: Ergebn. d. inn. Med. u. Kinderh. *1*: 157 (1908).
371. G. L. Rohdenburg & F. Prime: Arch. Surg. *2*: 116 (1921).
372. S. L. Warren: Am. J. Roentgenol. *33*: 75 (1935).
373. S. Morimoto: Acta dermat. *22*: 97 (1933).
374. B. Mendel: Klin. Wchnschr. *7*: 457 (1928).
375. N. Henning & H. Habs: Klin. Wchnschr. *8*: 1854 (1929).

cellular permeability, underlying these observations, might also be influenced by other factors. More recently, it has been reported that when tumor cells were incubated at body temperature for twenty-four hours in a saline solution, or in plasma, the transplantability was lost in the first case only, and it was assumed that the destruction of the tumor cells was a consequence of autolytic processes, which were prevented by the presence of plasma.[376]

With respect to the exposure to low temperatures, a differentiation must be made between experiments which determined the growth rate of neoplastic tissue cultures, and those testing·the subsequent transplantability to living animals, because freezing of Rous sarcoma,[377] or of rat and mouse tumors,[378] impeded the growth of explants in vitro, but not of transplants in vivo. It may be assumed that the lowering of the temperature did not cause an irreparable cellular damage, but merely induced a temporary latent period of growth, whereas growth was resumed after the transfer of the tumor material into a more favorable medium, i.e., the living organism. The fact that animal tumors could be successfully inoculated after exposure to extremely low temperatures has been corroborated in numerous studies;[379–385] in some experiments temperatures as low as minus 196° C were used,[381] and it was believed that this amazing ability to survive was connected with the presence of especially cold resistant "small

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376. H. Auler: Ztschr. f. Krebsforsch. *44*: 332 (1936).
377. C. Simonin: Compt. rend. Soc. de biol. *107*: 1029 (1931).
378. W. Cramer: Lancet *1*: 133 (1930).
379. M. Borst: Ztschr. f. Krebsforsch. *44*: 145 (1936).
380. O. Hörner: Verhandl. d. deutsch. path. Gesellsch. *30*: 338 (1937).
381. J. Klinke: Ztschr. f. Krebsforsch. *46*: 436 (1937); Growth *3*: 169 (1939).
382. H. Auler & B. Hohenadel: Ztschr. f. Krebsforsch. *48*: 179 (1938).
383. C. Breedis, W. A. Barnes & J. Furth: Proc. Soc. Exper. Biol. & Med. *36*: 320 (1937).
384. C. Breedis & J. Furth: Science *88*: 531 (1938).
385. K. Höfer, F. Holtz & M. Koinzer: Naturwiss. 1939: 275.

cells" in the neoplasms.[379] Again, it has been observed that, after freezing tumors for seven days at minus 35° C, transplants to animals resulted in tumor growth, in spite of the failure of this material to grow in tissue cultures.[385] Another author even subjected tissue cultures of Ehrlich mouse carcinoma to freezing at minus 196° C, and still noted survival of cells, although the growth was both delayed and retarded.[386] Considering these findings, it is questionable whether the extreme resistance of Rous sarcoma against freezing and subsequent thawing is actually incompatible with the assumption of a living agent as being responsible for this tumor, as has been contended [387] (cf. Chapter IX).

The mentioned sensitivity of malignant tumors to thermal effects gave rise to several studies aimed at therapeutic attempts, and it was reported that after heating rat tumors to 44–51° C by means of locally applied high frequency currents, regressions occurred in about fifty per cent of the cases.[388] In this connection it seemed significant to the authors of this study that heat application in vitro impaired the glycolysis of tumors more strongly than it impaired their respiration. Tail tumors of mice could also be made to disappear in some instances, following heating to 42–46° C,[389] and especially favorable effects were seen in analogous experiments, in which diathermy, causing a rise of the temperature in the tumors to 42° C, was combined with roentgen radiation.[390] The increasing of the body temperature of tumor-bearing mice to 44.1° C, achieved by keeping the animals for twenty minutes in a thermostat, also resulted in a complete loss of the transplantability of their tumors, but it must be remarked

386. K. Herzog: Ztschr. f. Krebsforsch. *50*: 412 (1940).
387. J. K. Miller & H. E. Eggers: Am. J. Cancer *23*: 94 (1935).
388. N. Westermark: Scandinav. Arch. f. Physiol. *52*: 257 (1927).
389. K. Overgaard: Acta radiol. *15*: 89 (1934).
390. K. Overgaard: Ugeskr. Laeg. 1935, 335; abstr. Ztschr. f. Krebsforsch. *42*: Ref. 132 (1935).

that the animals survived this drastic treatment for only eight hours at the most.[391] Thermal effects of a lesser degree, however, did not seem capable of successfully suppressing tumor growth, since artificial fever, resulting in a body temperature of 41° C, or external heat application, brought about only a temporary growth retardation and reduction of the size of metastases, in both animal experiments and in a few clinical cases.[372] Likewise, maintaining tumor-bearing animals in overheated receptacles did not appreciably inhibit the neoplastic growth.[392] A certain difference in the thermal sensitivity of various animal tumors has been demonstrated by a study in which the effects of short radio waves on Jensen rat sarcoma and on Walker rat carcinoma in vivo were measured. Although fifty per cent of tumor regressions occurred in the Jensen sarcoma after heating to 47° C for twenty-five minutes, it was necessary to apply the same quantity of heat to Walker carcinoma for forty-five minutes in order to achieve the same result; or exposure to 43.5° C for three hours had the same effect in Jensen sarcoma as a six hour exposure in Walker carcinoma. In vitro, however, both tumor types exhibited the same heat sensitivity, i.e., death resulted from the exposure to 47° C for less than twenty minutes.[393] It may be stressed again here that these experiments furnish additional proof for the assumption that the administration of short waves affects the tumor process basically by virtue of the heat production. A somewhat different aspect must be assigned to an investigation dealing with the influence of high temperatures on the development of spontaneous mouse tumors; in this study it was observed that in a strain with a spontaneous tumor incidence of about fifty per cent, a lesser tumor frequency and a delayed tumor growth resulted when

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391. G. Walker: Am. J. Cancer *25*: 301 (1935).
392. W. H. Woglom: Am. J. Cancer *21*: 604 (1934).
393. H. J. Johnson: Am. J. Cancer *38*: 533 (1940).

the animals were kept in a hot room (90–91° F), as compared with the relations in animals living at a temperature of 65° F. In explanation of these findings, it was stated that, when the loss of body heat is suppressed, the combustion level in the organism and the normal as well as the malignant growth rate are reduced at the same time, and the author believed that a statistical analysis of climatic factors would indicate similar relations regarding the frequency of human neoplasia in different geographic zones;[394] but it is obvious that other factors also will be of decisive influence in this respect.

On the other hand, within the last years, the exposure to low temperatures has been recommended, and has been tried as a therapeutic measure in malignant diseases, both in the form of local cold applications, and by artificially lowering the whole body temperature (general refrigeration). This trend was initiated by observations of a retarding influence of low temperature on the development of chick embryos,[395] and by measurements of skin temperatures in humans, suggesting an inverse relation between temperature and frequency of tumor development in various body segments.[396] The authors devised a method for reducing the body temperature of cancer patients to a temperature as low as 81–90° F (rectal) for a period up to three days,[396, 397] and reported very encouraging results in some cancer cases—reduction of tumor size, and sometimes complete disappearance of neoplasms or metastases, in which instances the tumor destruction was substantiated by histologic examinations. Striking relief of severe pains was noted following local or general refrigeration, as evidenced by an analysis of sixty patients treated by this method,[398] and an exact study on the influences of general hypothermia on metabolic processes has been presented.[399] Although only a short time has elapsed since the introduction of this therapy, several clinical studies have been recorded on pertinent experiences, but most of the results do not appear to be as favorable as the original reports. By means of local refrigeration, two advanced vesical cancers were cured, but in two other cases the patients succumbed to the disease.[400] Another author saw in the relief of pain the most outstanding benefit of hypothermia.[401] But other workers collected even less promising experiences with the treatment,[402–404, 404a] or stressed the complicating incidence of fatal pneumonia.[405] In contrast to the original communication, no histologic changes were found to result in the neoplasms from refrigeration,[403] or were observed only after local refrigeration.[405] Nor have animal experiments furnished convincing evidence for the possibility of successfully combatting neoplasia by means of low temperature. A growth retardation of sarcoma 180 has been noted in mice living at subnormal temperatures, but this effect might be explained by the great loss of body weight occurring.[406] Hibernation of mice with sarcoma 180 for seven hours on five successive days, lowering the body temperature below 20° C, caused only a temporary growth cessation, which was considered as being due to the caloric restriction, but no permanent effect resulted from this measure.[407] Among 95 mice with sarcoma 180, which were subjected to general refrigeration for from eight to forty-eight hours at a temperature of 5–7° C, only five tumor regressions occurred. These were interpreted as depending merely on the general health impairment, resulting from the drastic procedure, and it seems especially noteworthy that in vitro determinations of the metabolic rate in treated tumors did not reveal any change of this quality.[408] Hence it cannot be expected that even an energetic lowering of the body temperature may represent a promising therapeutic measure in neoplasia, a view which seems supported by the experimental observations discussed above and clearly pointing to an increased resistance of neoplastic cells against freezing, or against low temperatures. The proposed therapy, however, especially in the form of local cold application, may prove valuable in combatting cancer pains, and in this respect it must be mentioned that local application of dry ice (solid CO_2) has been shown as having beneficial effects on external tumors (tar cancer of mice,[409] human skin cancer,[410] and Paget's disease [411]). Furthermore, it must be admitted that the development of general hypothermia has led to highly interesting observations of a hitherto unknown nature in regard to cell physiology and metabolism.

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[394] R. H. Fuller, E. Brown & C. A. Mills: Cancer Research *1*: 130 (1941).

[395] L. W. Smith: Arch. Path. *28*: 420 (1939).

[396] T. Fay & G. C. Henny: Surg., Gynec. & Obst. *66*: 512 (1938).

[397] L. W. Smith and T. Fay: J. A. M. A. *113*: 653 (1939).

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[398] L. W. Smith: Arch. Path. *30*: 424 (1940).
[399] L. W. Smith & T. Fay: Am. J. Clin. Path. *10*: 1 (1940).
[400] A. McCravey: N. Y. State M. J. *40*: 1435 (1940).
[401] J. H. Talbot: New England J. Med. *224*: 281 (1941).
[402] A. M. Vaughn: J. A. M. A. *114*: 2293 (1940).
[403] J. C. A. Gerster et al: Bull. N. Y. Acad. Med. *16*: 312 (1940).
[404] A. J. Jones, J. Graham & A. Mueller: Am. J. Surg. *52*: 14 (1941).
[404a] J. B. Herrmann: Connecticut M. J. *5*: 721 (1941).
[405] N. M. Alter: J. M. Soc. New Jersey *37*: 586 (1940).
[406] F. Bischoff & M. L. Long: Am. J. Cancer *35*: 86 (1939).

Actinic energy. A connection between intensive solar radiation and cancer formation has for a long time been recognized as existing, based on the clinical evidence of the frequent development of precancerous dermatoses (xeroderma pigmentosum), and of skin cancers in persons exposed to strong sunlight for prolonged periods of time.[cf. 412–414] It was especially emphasized that skin cancers most frequently arise in body parts free of clothing, which are thus exposed to actinic energy, and that the tumor development was preceded by a cholesterol augmentation in these areas.[415] The significance attributed to this chemical change and divergent opinions on the subject have been discussed in chapter II. In imitation of human pathology, it has been possible to induce malignant tumors in animals by means of prolonged exposure to sunlight[416–418] and the development of spindle cell sarcomas was most frequently observed.[416] Another investigator, however, found the stimulus of solar radiation to be not very carcinogenic, but achieved neoplastic growths in white mice after the injection of photosensitizing substances (eosin, hematoporphyrin).[419] Studies devoted to the influence of sunlight or artificial light on animal tumors led to somewhat conflicting results. On the one hand, it was reported that spontaneous tumors developed more frequently in mice living in cages directly exposed to sunlight,[420] and it was claimed by the same author that different wave lengths of the visible spectrum exerted an inhibitory, or promoting influence, respectively, on the growth of transplanted carcinomas and sarcomas.[421] These observations, if substantiated, might

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[407] F. Bischoff & M. L. Long: Am. J. Cancer *39*: 241 (1940).

[408] A. Goldfeder: Cancer Research *1*: 220 (1941).

[409] S. M. Leitess: Ztschr. f. Krebsforsch. *38*: 513 (1933).

[410] A. A. Epstein: Arch. f. klin. Chir. *175*: 344 (1933).

[411] R. F. Wernicke & J. M. Spilzinger: Rev. Assoc. méd. argent. *51*: 631 (1937).

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[412] M. Larabi: Rev. de path. comparée *32*: 757; 853 (1932); abstr. Am. J. Cancer *23*: 846 (1935).

[413] E. Jascalevich: Libro de Oro, Buenos Aires, p. 1791 (1935).

[414] H. P. Morris & J. W. Thompson: J. Nat. Cancer Inst. *1*: 397 (1940).

[415] A. H. Roffo: 1st Internat. Cancer Congress, Madrid, *2*: 421 (1933).

[416] A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *12*: 529 (1935).

[417] E. G. Velloso: Libro de Oro, Buenos Aires, p. 1746 (1935).

[418] L. J. Sagasta: Libro de Oro, Buenos Aires, p. 1949 (1935).

[419] W. Büngeler: Ztschr. f. Krebsforsch. *46*: 130 (1937).

[420] A. de Coulon: Compt. rend. Soc. de biol. *91*: 280 (1924).

[421] A. de Coulon: Compt. rend. Soc. de biol. *90*: 445; 448 (1924); Arch. de physique biol. *3*: 223 (1924).

depend on an unequal absorption of the different wave lengths of light by the skin pigments, and it was reported that the longest wave lengths caused a growth inhibition of Ehrlich carcinoma and of tar cancer of mice.[422] Diffused daylight was seen to enhance the growth of inoculated tumors, if compared with tumor-bearing animals kept in darkness,[423] but another author failed to notice such a difference.[424] The statements that the tumorigenic action of tar [425] or of benzpyrene [426] in mice was enhanced by exposure of the animals to light also do not appear to be substantiated by several other investigations. No stimulation of tar cancer formation was seen in animals exposed to light—indeed, the animals excluded from light even yielded a greater number of tumors.[427] Benzpyrene treatment of mice resulted in approximately the same amount of tumors, regardless of whether the animals lived in light or dark cages.[428] In an extensive study reviewing the previous communications, it was found that tumor formation in mice by means of benzpyrene or cholanthrene was not promoted by exposure to light; on the contrary, a stronger dermatitis developed in those animals than in animals kept in darkness, and the number of tumors produced was reduced.[429] Likewise, by dividing mice painted with benzpyrene into two groups, one of them kept in complete darkness,

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422. C. Tedeschi: Pathologica *24*: 94 (1932); abstr. Ztschr. f. Krebsforsch. *37*: Ref. 46 (1932).

423. N. Soboleva & G. Schor: Vestn. Rentgenol. *7*: 281 (1928).

424. A. Sintini: Pathologica *25*: 336 (1933); abstr. Am. J. Cancer *23*: 128 (1935).

425. F. Vlès, A. de Coulon & A. Ugo: Arch. de physique biol. *12*: 255 (1935).

426. J. Maisin & A. De Jonghe: Compt. rend. Soc. de biol. *117*: 109 (1934).

427. M. G. Seelig & Z. K. Cooper: Libro de Oro, Buenos Aires, p. 1313 (1935).

428. J. Taussig, Z. K. Cooper & M. G. Seelig: Surg., Gynec. & Obst. *66*: 989 (1938).

429. I. Domiach & J. C. Mottram: Am. J. Cancer *39*: 234 (1940).

the other exposed to strong artificial light (free of ultra-violet rays), a tumor incidence of 79 per cent resulted in the first group, as compared with only 44 per cent in the second, and this finding was believed perhaps to depend merely on the better health condition exhibited by the mice of the first group.[430] By repeating these experiments with mice of a different pure strain, the authors confirmed the earlier results but, on the basis of the more recent observations, they did not consider it likely that an impaired nutritional status was responsible for the delayed and reduced tumor development in animals exposed to light.[430a] Likewise no definite explanation can be offered for the observation of a growth inhibition of transplanted rabbit sarcomas, following exposure to visible light, to infra-red, or to ultra-violet rays, although the authors of this study reported a proliferation of reticulo-endothelial elements as simultaneously resulting from these treatments, and therefore considered an activation of this system as being responsible for the effect mentioned.[431] Experiments dealing with tissue cultures showed neoplastic cells to react to light exposure with a stronger decrease of growth than normal cells.[432] Yet it is questionable as to what extent this effect is dependent on the optical, or on the thermal, components of light. It is certain that the latter was principally involved in the alleged cure of a single case of human breast cancer after exposure to sunlight, concentrated by lenses;[433] there is no doubt that the intensive heat production was responsible for the fortunate outcome of this unique procedure. But the existence of a more specific involvement of the action of solar

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430. J. J. Morton, E. M. Luce-Clausen & E. B. Mahoney: Am. J. Roentgenol. *43*: 896 (1940).

430a. J. J. Morton, E. M. Luce-Clausen & E. B. Mahoney: Cancer Research *2*: 256 (1942).

431. Y. Esaki: Jap. J. Obst. & Gynec. *19*: 358 (1936).

432. A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *9*: 512 (1932).

433. W. Becker: Ztschr. f. Krebsforsch. *21*: 253 (1924).

radiation on the frequency and distribution of neoplastic diseases has been assumed on the basis of extensive statistical analyses, which showed that a preponderance of skin cancer in certain populations, which are exposed to extensive sunlight because of geographical or occupational factors, is associated with a marked decrease of internal tumor frequency, if compared in an adequate manner with the proportions apparent in other population strata.[434, 435] Although a more thorough interpretation of this phenomenon, as well as a discussion of divergent statements, is given in chapter IX, it will be mentioned here that another author, who likewise pointed out a diminished incidence of internal tumors in hot climates, saw in this circumstance the expression of a real cancer immunity produced by the stimulus of solar radiation, and he considered the prevalence of skin cancer in these populations as a coincidental factor only.[436] Finally, reference is made to experiments demonstrating a sensitization against the light of the visible spectrum, occurring in mice painted with carcinogenic substances (tar, benzpyrene, etc.).[437]

Ultra-violet rays are—at least partially, perhaps mainly—responsible for the carcinogenic action of solar radiation, which action has been clearly shown from the results of animal experimentation. Since the first report on the production of malignant tumors in white mice by means of ultraviolet irradiation, continued for eight months,[438] numerous analogous findings have been reported. Neoplastic growth has been induced by similar methods in the skin of rats,[439, 440]

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[434] S. Peller: Lancet *2*: 552 (1936); Am. J. Hyg. *29*: 34 (1937).

[435] S. Peller & C. S. Stephenson: Am. J. M. Sc. *194*: 326 (1937).

[436] F. L. Apperly: Cancer Research *1*: 191 (1941).

[437] I. Domiach & J. C. Mottram: Nature, London *140*: 588 (1937).

[438] G. M. Findlay: Lancet *2*: 1070 (1928).

[439] W. Putschar & F. Holtz: Ztschr. f. Krebsforsch *33*: 219 (1930); Libro de Oro, Buenos Aires, p. 1103 (1935).

[440] A. H. Roffo: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *10*: 417 (1933); 2nd Intern. Cancer Congress, Brussels, *1*: 71 (1936).

in the skin of mice,[441, 442] and in rat eyes.[443] Similarly, as in the instance of solar radiation, an accumulation of cholesterol has been reported as occurring prior to the tumor development in body parts, irradiated with ultra-violet,[444] and it was furthermore claimed that ultra-violet rays, or sunlight, would bring about certain molecular changes in the cholesterol, indicated by the appearance of a fluorescence; [445] in another study, however, this photoactivity has been recognized as being only a pseudo-effect, caused by the formation of peroxides in the presence of moisture.[446] It is of great interest for human pathology that workers, who were able to produce malignant tumors in white rats by means of ultra-violet radiation, have stressed the fact that the doses necessary for achieving this effect were so high that any fears may be allayed regarding a similar occurrence as a consequence of the widespread use of therapeutic or cosmetic ultra-violet administration to humans.[447] The tumor development in animals following ultra-violet irradiation has recently been analyzed in very careful studies as to the dosage and the time required. Albino mice were found to be more susceptible than black ones,[448] and the wave lengths of 2900–3300 A were proved to be most carcinogenic.[449] Extensive investigations were recently performed by a group of authors who exposed male mice to ultra-violet radiation, employing devices which permitted

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441. C. W. Herlitz, I. Jundell & F. Wahlgren: Acta pediat. *10*: 321 (1931); abstr. Ztschr. f. Krebsforsch. *35*: Ref. 46 (1932).
442. F. Wahlgren: Zentralbl. f. Path. *60*: 102; 125 (1935).
443. K. Huldschinsky: Deutsche med. Wchnschr. *59*: 530 (1933).
444. A. H. Roffo: Ztschr. f. Krebsforsch. *41*: 448 (1935).
445. A. E. Roffo: Bol. inst. de med. exper. para el estud. y trat. d. cáncer *13*: 337; 369 (1936).
446. H. E. Staveley & W. Bergmann: Am. J. Cancer *30*: 749 (1937).
447. H. H. Beard, T. S. Baggess & E. von Haam: Am. J. Cancer *27*: 257 (1936).
448. H. P. Rusch & C. A. Baumann: Am. J. Cancer *35*: 55 (1939).
449. H. P. Rusch, B. E. Kline & C. A. Baumann: Arch. Path. *31*: 135 (1941).

exact measurements of the energy administered. It was found that the tumor production depended rather upon the total quantity than upon the intensity of the radiation, since higher doses affected the appearance of tumors only to a slight degree, whereas the latent period was markedly lengthened in animals receiving smaller radiation doses.[450] In dealing with the pathology of the tumors arising after ultra-violet radiation, it was stated that for the most part they represented fibrosarcomas, while combinations of fibrosarcoma and squamous carcinoma were less frequently found and squamous carcinomas found only rarely.[451] These observations are in apparent contrast to the relations encountered in human pathology where solar (ultra-violet) radiation results mostly in carcinomatous tumors. This discrepancy was satisfactorily explained by a study in which the penetration of ultra-violet radiation through separated mouse skin was measured. Wavelengths shorter than 3200 Å—only these are carcinogenic—were found to pass through the epidermis and to be absorbed by the dermis of the mouse tissue. Consequently, sarcomatous tumors arise in mice, since the main impact of the radiation is taken up by mesenchymal tissues, whereas in human beings a greater amount of radiation is absorbed by the epidermis, thus leading to epithelial tumors.[452] It is noteworthy that tumor formation has been accomplished in mice by means of the application of small quantities of tar, together with sub-threshold doses of ultraviolet light, which stimuli separately would not have led to neoplastic growth.[453] The supposition, however, that the tar cancer formation necessarily required the synergistic action of the ultra-violet part of light [454] has been contested, since it was shown that tar cancer also developed in mice when the animals were excluded from the action of light.[455] The growth of Ehrlich mouse carcinoma was found to be enhanced by ultra-violet irradiation, and it was assumed that a histamine production, resulting from this treatment, was responsible for this effect.[456] In contrast to the action of visible light of longer wave lengths, ultra-violet rays did not impede the growth of neoplastic tissue cultures,[457] but did exert such an influence only after the addition of photosensitizing substances such as eosin and erythrosin.[458] In this connection, it is recalled that ultra-violet irradiation of skin cancers has been found to be an effective therapeutic measure, when preceded by a similar photoactivation,[459] as detailed in chapter II.

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[450] H. F. Blum, J. S. Kirby-Smith & H. G. Grady: J. Nat. Cancer Inst. 2: 259 (1941).

[451] H. G. Grady, H. F. Blum & J. S. Kirby-Smith: J. Nat. Cancer Inst. *2*: 269 (1941).

[452] J. S. Kirby-Smith, H. F. Blum & H. G. Grady: J. Nat. Cancer Inst. 2: 403 (1942).

[453] E. Dormanns: Ztschr. f. Krebsforsch. *40*: 577 (1934).

Acoustic energy and its influence on neoplasms have been studied in but one investigation, in which ultrasonic vibrations were applied to Walker rat sarcoma 319, and it was observed that these waves penetrated the tumors, causing a production of heat and consequently extravasation, but only an uncertain growth inhibition was noted, while determinations of the metabolism of treated tumors indicated a slight stimulation of the glycolysis.[460]

Mitogenetic radiation. If a discussion of this phenomenon and its relations to neoplasia is entered into here, this is done with full consciousness of the fact that their experimental

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454. W. Büngeler: Klin. Wchnschr. *16*: 1012 (1937).
455. O. Teutschländer: Klin. Wchnschr. *16*: 1284 (1937).
456. F. Ellinger: Fundament. radiol. (Berlin) 4: 181 (1939).
457. A. H. Roffo: Bol. inst. med. exper. para el estud. y trat. d. cáncer *10*: 209 (1933).
458. A. H. Roffo: Bol. inst. med. exper. para el estud. y trat. d cáncer *7*: 338 (1931).
459. A. Jesionek & H. von Tappeiner: Deutsches Arch. f. klin. Med. *82*: 223 (1905).
460. F. F. Beck & J. C. Krantz, Jr.: Am. J. Cancer *39*: 245 (1940).

and theoretical bases, as expanded by Gurwitsch, their discoverer,[461] have by no means found full recognition, but instead represent a much debated problem, still far from a definite elucidation. On the other hand, a great number of findings and statements have been forthcoming which touch on cancer pathology, and therefore it seems appropriate to present them here as contributions, the value of which it will be possible to appraise scientifically only at a future date. As indicated by its name, the mitogenetic radiation has been assumed to represent an energy of defined physical qualities, which emanates from living cells during the process of division. The various methods for its detection and determination are mainly based on the principle that this radiation, it is thought, stimulates the mitotic processes in other living cells which are exposed to it. For this purpose—involving the counting of mitoses in "irradiated" and in control material—yeast cultures or plant roots have mostly been used. The source of this energy was believed to be derived from energetic enzymatic processes taking place in the dividing cell. While wave lengths of 1900–2500 Å generally were assigned to this radiation, it was also thought possible to differentiate various spectral parts according to the specific enzymatic process from which they originate. A close connection with neoplastic growth seemed to be given by the fact that these tissues excel in mitotic activity, and, in fact, several workers on this problem reported the presence of a very strong mitogenetic radiation in malignant tumors of humans, or of animals.[462–465] In addition to a quantitative increase, quali-

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461. A. Gurwitsch: Die mitogenetische Strahlung, Springer, Vienna 1932.

462. A. Gurwitsch & L. Gurwitsch: Ztschr. f. Krebsforsch. *24*: 220 (1923).

463. T. Reiter & A. Gabor: Sonderh. d. Siemenskonzern 1928; quot. 461.

464. M. Kisliak-Stakewitsch: Ztschr. f. Krebsforsch. *29*: 214 (1929).

465. Sh. Odani: Jap. J. Obst. & Gynec. *20*: 100 (1937).

tative differences from the behavior of normal tissues have also been claimed for the mitogenetic radiation of tumors. While normal tissues should generally exhibit only a "secondary" radiation, due to the circulating blood in the organs, the mitogenetic radiation of neoplasms should primarily originate and be present not only in the cellular elements but also in the interstitial humoral media or, in vitro, in the surrounding medium, which circumstances were considered to be indications of a diffusibility of the responsible factors from the malignant cells, on account of their changed permeability. In intact cancer tissue the mitogenic radiation was only detectable after the addition of glucose, and was supposed to originate from glycolysis, whereas ground neoplastic tissue also exhibited a mitogenetic radiation in sugar-free medium, which phenomenon was thought to be due to proteolysis. These assumptions appeared to be substantiated by the characteristic spectra, established for the respective enzymatic processes.[466, 467] In vivo, it was thought, both sources of radiation would occur in malignant tumors, with a prevalence of glycolysis in proliferating, intact cells, and a predominance of proteolysis in necrotic, regressive areas. Since either no, or only a weak, radiation was observed in the papillomas arising from tar application, and the increase coincided with the onset of malignancy, a direct association between these two phenomena has been assumed to exist.[468] The presence of mitogenetic radiation in animal tumors was believed also to be provable by means of a physico-chemical method, viz., by the deformation of Liesegang's rings.[469]

A great significance, furthermore, has been attributed to the finding that the blood of cancer patients, or of animals

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466. A. Gurwitsch & L. Gurwitsch: Biochem. Ztschr. *196*: 267 (1928).
467. L. Gurwitsch: Biochem. Ztschr. *236*: 425 (1931).
468. Salkind & Schabad, quot. 461.
469. A. H. Roffo: Bol. inst. med. exper. para el estud *y* trat. d. cáncer *11*: 17 (1934).

soon after tumor inoculation, possessed no, or a diminished, mitogenetic activity, while such mitogenetic activity was found in blood of tumor-free persons or animals.[466, 470–474] In spite of the fact that, in addition to neoplastic conditions, severe anemias were also found accompanied by a loss of the mitogenetic radiation in the blood,[475–477] a diagnostic and prognostic use has been recommended, and special techniques for this test have been devised.[478, 479] It seemed of particular significance that the non-radiating cancer blood was also capable of extinguishing the mitogenetic radiation of normal blood, or of other radiating sources,[468, 480, 481] and a diagnostic utilization of this phenomenon was thought promising. But at the same time reference must be made to studies in which either an increased mitogenetic radiation of cancerous blood was observed[482] or no difference in the growth of yeast cultures was found, when under the influence of blood or tissue extracts of normal, or of tumor-bearing animals, respectively. These findings, naturally, evoked the greatest doubt regarding the phenomenon of mitogenetic

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470. L. Gurwitsch & S. Salkind: Biochem. Ztschr. *211*: 362 (1929).

471. W. W. Siebert: Biochem. Ztschr. *226*: 253 (1930).

472. J. Blacher, L. D. Losner & A. P. Tschmutowa: Verh. d. 1. internat. Kongr. f. Elektro-Radio-Biol. *2*: 765 (1935); abstr. Ztschr. f. Krebsforsch. *43*: Ref. 70 (1936).

473. O. E. Nudolskaja: Arch. f. Gynäk. *163*: 30 (1936).

474. Sh. Odani: Jap. J. Obst. & Gynec. *20*: 109 (1937).

475. H. Gesenius: Biochem. Ztschr. *226*: 257 (1930).

476. J. S. Klenitzky: Arch. biol. nauk *35*: 3; 213 (1935); abstr. Ztschr. f. Krebsforsch. *43*: Ref. 70 (1936).

477. G. Salvadori-Paleotti: Verh. d. 1. internat. Kongr. f. Elektro-Radio-Biol. *2*: 950 (1935); abstr. Ztschr. f. Krebsforsch. *43*: Ref. 70 (1936).

478. M. Heinemann: 1st Internat. Cancer Congress, Madrid *1*: 200 (1933).

479. M. Heinemann & R. Seyderhelm: Arch. biol. nauk *35*: 107 (1934); abstr. Ztschr. f. Krebsforsch. *43*: Ref. 69 (1936).

480. W. W. Siebert & H. Seffert: Biochem. Ztschr. *289*: 292 (1937); *302*: 301 (1939).

481. A. Gurwitsch & L. Gurwitsch: Enzymologia *5*: 26 (1938).

482. J. E. Morelli: Acta Cancrol. *2*: 93 (1936).

radiation in general.[483] In conjunction with the extinction of mitogenetic radiation, ascribed to the blood of cancer patients, some experiments were performed with luminous bacteria, indicating that this radiation, too, might be inhibited by cancerous blood; the authors, however, did not come to any final conclusions.[480] A finding of related nature has been obtained by other authors, who found that the serum of cancer patients diminished the fluorescence of uranine solutions to a higher degree than the sera of cancer-free persons,[484] but the same authors found, in this respect, no difference between the sera of normal mice and of animals with tar cancer.[485]

No detailed account can be given in this book of the *roentgen radiation,* and of the related physical energies emitted by radioactive substances, as has already been stated in the first chapter. There it was also emphasized that these radiations not only represent one of the most valuable therapeutic procedures in neoplasia but that they must at the same time be considered also as powerful carcinogenic stimuli; tentative explanations of this fact have been offered, and will be further elaborated in chapter VIII. Short reference is made, in connection with these radiations, to investigations of probably far reaching general significance, recently performed on *neutron radiation* originating from destructive actions upon the atomic structure of various elements. A preliminary report has been given on twenty-four patients with far advanced malignancy, treated with fast neutron radiation. In the course of this study, a decrease of the size of tumors was seen in some of the cases treated, and eight patients survived one year after the start of the

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483. W. Luther: Ztschr. f. Krebsforsch. *49*: 404 (1939).

484. A. Boutaric & J. Bouchard: Compt. rend. Soc. de biol. *118*: 1188 (1935).

485. A. Boutaric & J. Bouchard: Compt. rend. Soc. de biol. *120*: 293 (1935).

therapy, although still affected with neoplasia.[486] It is obvious that these experiments can be considered only as a starting point, and that much greater experience is required before any attempt can be made to evaluate this procedure. A different approach has been chosen in animal experiments, by means of the application of slow neutron radiation to mouse sarcoma 180 into which previously lithium or boron had been injected; because of the secondary radiation, due to the concentration of these elements in the tumors, the treatment strongly affected the neoplasms, causing regression, without damaging the normal tissues.[487, 488] These experiments were, however, not considered wholly satisfactory by the authors themselves; they stressed that for practical purposes it would be necessary to achieve a greater differential between the radiation effects in normal and in neoplastic tissues than has thus far been possible.[489]

Summary.

The material which has been surveyed in this chapter may be, from one point of view, divided roughly into two categories: The first includes a great number of presumably established findings, which have been obtained by physicochemical methods and confirm, supplement or modify data which have resulted from other approaches, such as inorganic or organic analysis and determinations of enzymatic or metabolic processes. Observations of this nature have been encountered in the discussion of correlations between cellular permeability and inorganic and organic cell constitution; in the presentation of surface tension, with regard to the source of surface-active substances; in the problems of hydrogen ion concentration and of reduction-oxidation potential, with reference to the influence of tumor metabolism; and in the colloid chemistry of proteins, as supplementing the findings of chemical analyses. It is obvious that the value of these results is greatly heightened by their making it possible to secure confirmatory evidence, which is correspondingly less subject to errors of experimentation and of interpretation, especially in view of the fact, stated in the introduction, that physicochemical methods have the advantage of being frequently applicable to biological material in its natural state.

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[486] R. S. Stone, J. H. Lawrence & P. C. Aebersold: Radiology *35*: 322 (1940).

[487] P. G. Kruger: Proc. Nat. Acad. Sc. *26*: 181 (1940).

[488] A. Zahl, F. S. Cooper & J. R. Dunning: Proc. Nat. Acad. Sc. *26*: 589 (1940).

[489] P. A. Zahl & F. S. Cooper: Radiology *37*: 637 (1941).

On the other side, a great many facts have been recorded in this field which appear to represent original contributions towards an understanding of tumor pathology, by means of applying physicochemical interpretations to it. It is true, however, that some of the broader concepts, envisaging a supposed change or deviation of certain physicochemical qualities as being of primary significance for neoplasia in general, have been based on insufficient experimental data, or have even been made untenable as a consequence of additional investigation, such as has been especially demonstrated in the case of hydrogen ion concentration. Again it must be noted, as in the preceding chapters, that the physicochemical approach has also furnished strong evidence for a close correlation between phenomena observable in neoplasms with those known to be connected with rapid growth. In this respect it is referred to permeability, surface activity, and reduction-oxidation potential, and while no definite proof is available, it seems to be within the range of possibility that by these methods a differentiation between neoplastic and normal growth might be accomplished. For this purpose, it would seem to be of great importance to employ chemically induced neoplasms for pertinent studies, rather than the inoculated tumors generally used up to now. Likewise, in the realm of colloid chemistry several interesting observations which have been reported cannot be evaluated definitely, on account of their fragmentary character, which relate only to some phases or types of tumor pathology.

Highly interesting aspects have been opened up by some of the investigations of biophysical character, both with regard to carcinogenesis, due to certain physical stimuli, as well as in respect to phenomena which were believed to present diagnostic or therapeutic possibilities. In particular, attention is directed to the results of bioelectric measurements, to the sensitivity against thermal energy of neoplastic cells, as far as deviations from the reaction of normal tissue have been established, and to the apparently very effective carcinogenic stimulus of certain forms of actinic energy. In all these respects, it is to be expected that correlated work will be aimed at attempts to define the accompanying chemical changes in the tissues and, on the other hand, the use of physical methods for biological problems conforms with the general trend in science, which tends to reduce the qualitative phenomena, as exhibited in the chemistry of matter, to phenomena of quantity, as feasible in the realm of physics.

CHAPTER IV

ENZYMES

THE study of enzymes undoubtedly bears on very important problems of biology, for there is probably no other branch of biochemistry which more strongly characterizes the border separating living matter from inanimate matter than the science of enzymes. Starting from the most primitive microorganism up to the most highly organized forms of animal life, every living cell appears to be endowed with special mechanisms enabling it to perform complicated chemical processes of fission, synthesis, and conversion rapidly and in a specific manner which, outside of living substrates, require a much longer time and use of involved technical operations. Although the chemical isolation and definition of the enzymes responsible for this amazing quality of living matter has been accomplished only in a few instances, in most cases it has been possible to extract the active factors from cells and tissues and to study their kinetics in vitro. Very valuable information has been gained from such investigations, since they permit the determination of qualitative and quantitative changes of enzymatic activity which accompany physiological or pathological phenomena. Therefore, it is easily understandable that the tumor problem has undergone an extensive elaboration from the viewpoint of enzymology, both in the direction of analytical work which was expected to be helpful in elucidating the mechanism of tumor formation or in furnishing diagnostic methods and, also, possibly for the purpose of deriving measures of therapeutical value. As will be shown later, special circumstances induced oncologists to focus particular attention on certain enzymes in connection with the cancer problem but, in general, the viewpoint prevailed that very probably the change of a normal into a malignant cell might be associated with, or even caused by, deep-seated alterations of enzymatic properties of the cell. The following presentation will be grouped according to the usual classification of enzymatic factors, but it is stressed that for the sake of continuity the discussion of a number of enzymes intimately connected with the intermediate carbohydrate metabolism and with oxidation processes has been included in chapter VI dealing with the tumor metabolism. In general, it is obvious that a close interdependence exists between enzymes and metabolic processes, hence a rather arbitrary division of the material or an overlapping of the presentations can hardly be avoided.

1. *Proteolytic Enzymes.*

Proteolytic enzymes in malignant tumors. The phenomenon of invasive neoplastic growth which penetrates and destroys surrounding normal tissues, together with experimental findings which indicated an increased autolysis of tumors, led several early authors to assume that neoplasms possess proteolytic enzymes in increased quantities or of an increased activity.[1-4] Experimental work, however, was in most instances unable to corroborate this contention. As long ago as 1909 it was reported that no difference existed between normal and neoplastic tissues as to their proteolytic

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1. F. Blumenthal & H. Wolff: Med. Klin. *1*: 166 (1905).
2. C. Neuberg & E. Ascher: Arb. a.d. path. Inst. Bern 1906: 593.
3. F. Blumenthal, E. Jacoby & C. Neuberg: Med. Klin. *5*: 1595 (1909).
4. C. Neuberg & A. Gottschalk: Handbuch der Biochemie, Vol. *4*: 448; ed. 2, G. Fischer, Jena 1925.

activity.[5] Greater significance must be assigned to investigations performed with more modern methods of enzymology, principally developed by Willstätter. One of the older studies indicated certain peculiarities of a qualitative nature as being inherent in tumor proteinases. A pH of 7.0 was reported to represent the optimum for the proteolytic enzymes of human neoplasms and of Flexner-Jobling rat carcinomas; this would imply a tryptic nature of the proteinases tested. Furthermore, a marked inhibition of these enzymes by sodium and particularly by calcium salts was observed, which finding was used to explain the growth inhibition occurring after a treatment of tumor tissue with calcium salts prior to its inoculation[6] (cf. chapter I). A later study, however, definitely established a cathepsin nature for proteinases extracted from rat tumors, and a comparison of the tumor cathepsin with the cathepsin of normal rat liver showed the kinetics of these enzymes to be practically identical.[7] Tissue extracts were prepared from normal rat organs, Jensen rat sarcomas, normal human organs and from human neoplasms; determinations of the proteolytic activity were performed in these extracts, after the addition of adequate amounts of cysteine for the purpose of removing heavy metals and of insuring optimal conditions for the enzyme activity. The results of this study failed to disclose an abnormally great proteolytic activity of malignant tumors. The rat tumors occupied an intermediary position between spleen and liver of rats; some of the human neoplasms tested exhibited a strong proteolytic activity, but these values were also within the range of those obtained with normal human tissue.[8] Similarly, other workers were unable to detect any qualitative or quantitative differences between proteinases extracted from normal tissues and from human tumors. This conclusion was derived from extensive studies on the kinetics of the enzymes in question, which were tested by employing variations of the substrates, of the pH and of the dilution of the extracts.[9, 10] Likewise, investigations of proteinases present in glycerine extracts of Ehrlich mouse carcinomas gave no evidence for an increased activity, nor was an increased proteolysis observed when the enzymes acted upon tumor protein.[11] Other workers extended their studies not only to various animal tumors (mouse carcinomas, mouse sarcomas, fowl sarcomas), but also to the normal organs of tumor-free animals and of tumor hosts. The cathepsin content of tumors was found to be lower than that of normal organs, but the organs of tumor-bearing animals were richer in cathepsin than the organs of tumor-free animals, particularly in the case of the spleen and the liver. The tumor enzymes exhibited a degree of activation definitely below that of normal enzymes, and necrotic tumor parts were shown to contain less cathepsin than intact tumor areas.[12] The latter observation is in agreement with findings obtained with Brown-Pearce rabbit carcinomas; the cathepsin values of this tumor corresponded approximately to those of normal organs, but decreased with aging and development of necrosis in the tumor.[13] The finding of an increased cathepsin activity in the liver and other parenchymatous organs of tumor hosts has been corroborated by later work,[14, 15] and

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[5] L. Hess & P. Saxl: Beitr. z. Karzinomforsch. No. 1 (1909).

[6] K. G. Falk, H. M. Noyes & K. Sugiura: J. Biol. Chem. *53*: 75 (1922).

[7] K. G. Stern: Biochem. Ztschr. *234*: 116 (1931).

[8] H. A. Krebs: Biochem. Ztschr. *238*: 174 (1931).

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[9] H. Kleinmann & F. Werr: Biochem. Ztschr. *241*: 108; 140; 181 (1931).

[10] H. Kleinmann: Klin. Wchnschr. *10*: 1503 (1931).

[11] P. Rondoni: Biochem. J. *26*: 1477 (1932).

[12] E. Maschmann & E. Helmert: Ztschr. f. physiol. Chem. *216*: 161; *218*: 142 (1933).

[13] G. Mori: Acta dermat. *32*: 53; 57 (1938).

[14] A. Purr: Biochem. J. *28*: 1907 (1934).

[15] A. Rosenbohm: Biochem. Ztschr. *289*: 279 (1937).

one author[14] reported that the muscles of rats with transplanted tumors also showed a considerable proteolytic activity, in contrast to the muscles of normal animals which are almost free of this enzyme. The organs of rats in which tumor transplants had failed to grow were claimed to exhibit an especially high proteinase activity. However, these latter statements have been contested; that is, the liver of rats, resistant against inoculation of Jensen sarcoma, did not show any appreciable increase of cathepsin, and the muscles of rats, rabbits and fowl bearing transplanted tumors were found to be as devoid of cathepsin as the muscles of normal animals. The increase of cathepsin observed in parenchymatous organs of tumor hosts was believed most probably to represent a defensive mechanism for the purpose of removing products of tissue disintegration.[16] The fact that in one study, several human neoplasms were found completely lacking in proteolytic activity[17] may have been due to deviations in the technique of extraction used, whereas another report on a very high cathepsin content in a melanosarcoma of a horse[18] does not warrant any general conclusions. Clinical significance may be attached to the statement that sarcomatous extracts digested elastic fibers to a much greater degree than did carcinomatous extracts; this observation may be connected with the familiar fact that sarcomas possess an increased tendency to invade blood vessels and to metastasize in this manner.[19]

In more recent studies the cathepsin content of Ehrlich mouse carcinomas was found to be diminished when compared with normal parenchymatous organs. In contrast to the aforementioned statements, the cathepsin values in the organs of tumor-bearing mice were slightly lower than those found in animals free of tumors.[20] A method has been devised for isolating cathepsin from tissues by means of extracting and precipitating the globulin to which the enzyme is attached, and from which it can be freed by digestion. In this manner, cathepsin was prepared from normal rat liver and from Jensen sarcomas; both enzyme preparations were found to be capable of digesting heterogeneous proteins.[21] In recent work the proteinases of transplanted hepatic rat tumors were investigated, and this tumor type may be considered a particularly interesting subject for study, on account of its rapid growth and because it offers the opportunity of comparing its enzymic activity with that of the parent tissue, i.e., of normal rat liver. This comparison revealed that the cathepsin of the hepatomas, tested with hemoglobin as substrate, exceeded, both in quantity and in activity, the enzyme of normal and of regenerating rat liver.[22] This phenomenon was assumed also to be significant with regard to processes of protein synthesis in the tumor, since there is sufficient reason to believe that protein synthesis is brought about by the action of the same enzymes which split proteins (cf. later sections and chapter VI).

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[16] E. Maschmann & E. Helmert: Ztschr. f. Krebsforsch. *45*: 423 (1937).

[17] G. Vercellana: Ztschr. f. Krebsforsch. *43*: 163 (1936).

[18] A. Purr: Ztschr. f. Krebsforsch. *41*: 483 (1935).

[19] D. M. Tichomirov: Virchows Arch. f. Path. Anat. *292*: 310 (1934).

Divergent opinions have been expressed concerning the physicochemical state of tumor proteinases. Some authors reported that eighty to ninety per cent of the cathepsin present in tumors can be eluted (lyo-form), and hence tissue extracts should exhibit relations comparable to those in vivo.[12] Other workers, however, doubted that studies performed with tumor extracts furnished adequate information in this respect, since their experiments indicated that a con-

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20. M. J. Eisen & A. Langer: Enzymologia *2*: 321 (1938).
21. M. E. Maver: J. Biol. Chem. *131*: 127 (1939).
22. M. E. Maver, G. B. Mider, J. M. Thompson & J. W. Thompson: J. Nat. Cancer Inst. *2*: *277* (1941).

siderable part of the enzyme and especially of its activators were present in tumors in the desmo-form, which could not be separated from the cellular structure by various means of extraction.[23] This leads to another point of controversy, that is, to the problem of activators of tumor proteinases. The phenomenon that neoplastic tissue cultures cause a liquefaction of the protein-containing medium much more rapidly than do cultures of normal tissues was believed to be due to the presence of a thermostable activator of fibrinolysis.[24] It cannot, however, be assumed that this factor is generally active in malignant tumors, since an investigation failed to reveal the presence of such activators in the Ehrlich mouse carcinoma.[25] Great importance has been attributed to sulfhydryl compounds, particularly to the reduced form of glutathione, as activators of tissue proteinases, and the accumulation of reduced glutathione, claimed to occur in neoplasms (cf. chapter II), was regarded as the underlying cause for an increased proteolytic activity.[26] It must, however, be mentioned that other investigators did not find it possible to share this view as to the importance of glutathione for tumor proteolysis,[12] and one study demonstrated a diminution of sulfhydryl-containing activators of proteinases in Ehrlich mouse carcinomas.[20] On the other hand, a series of investigations presented definite evidence for the close connection of sulfhydryl compounds with proteolytic processes in normal and neoplastic tissues. The authors of these studies proceeded from the realization that, in addition to temperature, pH and enzyme concentration, the oxygen tension represents an important factor for proteolysis. Proteolytic extracts of Jensen sarcoma and of Walker carcinoma, as well

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23. E. Waldschmitz-Leitz & K. Bartunek: Ztschr. f. physiol. Chem. *233*: 36 (1935).
24. F. Demuth & I. Riesen: Biochem. Ztschr. *203*: 22 (1928).
25. L. Pozzi: Atti acad. Lincei *17*: 865 (1933).
26. E. Waldschmidt-Leitz & A. Schäffner: Naturwiss. 1930: 280.

as the digestion of coagulated egg white, fibrin or gelatin by papain, were tested under varying pH and oxygen tension. In all these instances, the proteolysis taking place under reduced oxygen tension exceeded the enzymic activity observed under atmospheric oxygen pressure. At the same time, tests for the presence of sulfhydryl groups indicated that conditions which favor the persistence of reduced —SH groups (acid pH and low oxygen tension) enhanced the proteolysis; on the other hand, destruction of the sulfhydryl groups by hydrogen peroxide, by aeration or by the addition of copper salts caused the proteolysis to disappear.[27] In continuation of this work, the rate of the proteolysis and the accompanying sulfhydryl concentrations were determined, in extracts of rat tumors and of normal rat tissues, with the following results. In the tumor extracts the rate of the protein disappearance and the rate of the increase of protein split products were much greater under reduced oxygen tension than under atmospheric oxygen tension. When employing reduced oxygen tension, the amount of sulfhydryl groups remained high during the first two hours of the experiment, whereas under atmospheric oxygen pressure a rapid decrease of the sulfhydryl groups occurred during the same length of time. Extracts of hepatic tissue of rats yielded results similar to those obtained with tumor extracts. Extracts of skeletal muscles of rats showed proteolysis only under reduced oxygen tension, but failed to do so under atmospheric oxygen pressure. In the experiments with muscle, the quantity of reduced sulfhydryl compounds remained unchanged during the first two hours regardless of whether atmospheric or reduced oxygen tension was employed, but a sharp drop of the sulfhydryl concentration took place after two hours, in experiments using atmospheric oxygen pressure.[28] Still greater significance may be assigned to results obtained in a later study, in which tissue extracts were examined during consecutive periods of anaerobic and aerobic conditions. Extracts of normal muscle (rat, rabbit) showed proteolysis when kept in a nitrogen atmosphere, whereas during a subsequent period of oxygenation a reversal of the proteolysis—i.e., protein synthesis—occurred, as manifested by a decrease of the amino nitrogen and an increase of the protein nitrogen; during this period of oxygenation and protein synthesis, a sharp decline of reduced sulfhydryl groups was observed. Essentially the same results were obtained with extracts of Jensen sarcomas and of Walker carcinomas. The following theoretical inferences were drawn from these observations: impaired blood supply and low oxygen tension in vivo lead to the accumulation of acids, resulting in an acid shift of the tissue pH and in the stabilization of reduced sulfhydryl groups; these conditions favor proteolysis. On the other hand, adequate oxygen supply and effective blood circulation insure complete combustion and removal of acids and thus tend to shift the sulfhydryl systems into the oxidized state; this condition favors protein synthesis. As far as cancerous tissue is concerned, these mechanisms may be responsible for the prevalence of proteolysis in some parts of the tumor and for the predominance of protein synthesis in others, and thus an explanation is available both for the growth and for the self-digestion of neoplasms.[29] More recent work indicated that a connection also exists between protein and carbohydrate metabolism, since the rates of proteolysis and glycolysis of normal and neoplastic tissues were seen to run parallel, and since proteolysis was activated by addition of lactates.[30] This has been confirmed by another worker who observed an increased proteolysis of Jensen sarcoma in vitro after the addition of small quantities of lactic acid; this substance caused an increase in reduced glutathione, which in turn is an activator of cathepsin and of methylglyoxalase, and therefore this mechanism was considered to be very important for tumor metabolism.[15]

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[27] C. Voegtlin & M. E. Maver: Pub. Health Rep. *47*: 711 (1932).

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[28] M. E. Maver, J. M. Johnson & C. Voegtlin: Pub. Health Rep. *48*: 42 (1933).

[29] C. Voegtlin, M. E. Maver & J. M. Johnson: J. Pharmacol. & Exper. Ther. *48*: 241 (1933).

Summarizing the studies on tumor proteinases recorded so far, the following conclusions may be offered: studies performed in vitro with cathepsin extracted from malignant tumors failed in general to give evidence for a qualitative or quantitative deviation of these enzymes from the normal; an exception is represented by the recently studied cathepsin of rat hepatomas, which was found to surpass the enzyme of normal liver both in activity and quantity.[22] The fact that clinical observations and phenomena apparent in neoplastic tissue cultures indicate that the proteolytic activity of malignant tumors may exceed that of normal tissues appears to be in conflict with the results of the studies performed with proteolytic tumor extracts. This discrepancy, however, has at least in part been explained as depending on factors which undergo variations in living tissues and decisively influence the proteolysis (pH, oxygen tension, sulfhydryl compounds). In addition, it is conceivable that, in consequence of the rapid dying of tumor cells, proteinases are constantly liberated from them and may act then upon the surrounding medium; in this manner, the impression of an increased proteolytic activity may be simulated in vivo, whereas quantitative measurements in vitro of the activity of enzymes extracted from tumors are not able to give evidence of such a phenomenon.

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30. W. M. Rubel: Biochem. Ztschr. *283*: 180 (1936).

Investigations on *peptidases* of malignant tumors were initiated a long time ago, but the results of these studies failed to disclose characteristic properties for neoplasms. Tumor extracts did not react in a manner deviating from the normal with glycyl-l-tyrosine, leucyl-glycyl-glycine and polypeptides prepared from silk fibroin.[31, 32] Sometimes, differences in the velocity of the reactions were noted, but these differences were not constant;[33] the absence of peptidases reported for cell-poor scirrhous tumors does not appear surprising.[32] Somewhat more promising aspects were derived from investigations with the tripeptide d-alanyl-glycyl-glycine; whereas extracts of normal organs caused a cleavage of alanine, in most cases of cancerous extracts a liberation of glycine was observed to occur first,[34] yet the latter finding was observed not in all, but only in a majority, of the tumors tested, and no further examinations of this enzymatic process have been announced. Quantitative estimations of the peptidase activity of tumor tissues, which were performed by employing glycyl-glycine and leucyl-glycine as substrates, showed that values are the lower limit of those obtained with normal organs,[35] and no characteristic change of ereptic enzymes (peptidases) was observed in uterine cancers.[36] According to an earlier investigation, metastases-free organs of tumor patients exhibited a decreased ereptic activity, but a similar phenomenon did not occur in mice bearing

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[31] E. Abderhalden & P. Rona: Ztschr. f. physiol. Chem. *60*: 415 (1909).

[32] E. Abderhalden, A. H. Koelker & F. Medigreceanu: Ztschr. f. physiol. Chem. *62*: 145 (1909).

[33] E. Abderhalden & F. Medigreceanu: Ztschr. f. physiol. Chem. *66*: 265 (1910).

[34] E. Abderhalden & L. Pincussohn: Ztschr. f. physiol. Chem. *66*: *277* (1910).

[35] S. L. Malowan: Ztschr. f. Krebsforsch. *37*: *277* (1932); Schweiz. med. Wchnschr. *63*: 336 (1933).

[36] K. Nakahori: Jap. J. Obst. & Gynec. *18*: 120 (1935).

transplanted tumors.[37] For the Brown-Pearce rabbit carcinoma, lower values of peptidases were determined than for normal liver or kidney; a further decrease accompanied the necrosis of the tumor, and only slight changes were noticeable in the organs of the tumor-bearing rabbits.[38] Investigations of recent date on various peptidases prepared from human and animal tumors did not yield uniform or characteristic results which might be used for an enzymic differentiation of neoplastic from normal tissue, but it was observed that enzyme solutions prepared from malignant tumors were less stable than those derived from normal tissues.[39] In a recent study, discussed previously, the peptidase activity of transplanted rat hepatomas was found to exceed that of normal rat liver.[22] As a consequence of the alleged occurrence of d-amino acids in neoplasms (cf. chapter II), attempts have been made to prove the presence of d-dipeptidases in tumors, but these studies yielded negative results for Jensen rat sarcoma[40] and other workers failed to detect any difference between this tumor and normal rat tissue; both failed to split peptides containing d-amino acids.[41]

In connection with tumor peptidases, it may be mentioned that an enzymatic activity deviating from the normal has been claimed to be exhibited by the stomach contents of patients with gastric cancer; in this neoplastic disease, gastric contents were reported to split glycyl-tryptophane after twenty-four hours of incubation, which may be recognized by a red color developing in the presence of free tryptophane after bromine addition, whereas gastric contents of cancer-free persons caused a fission of the dipeptide only after sixty-two to eighty hours.[42] However, some favorable experiences reported with this test [43, 44] are opposed by negative results of other investigators [45–47] and the scant usefulness of this procedure is reflected by the failure to resume its study more recently. In gastric contents of patients with gastric cancer, increased amounts of non-protein nitrogen and of amino acids have been found and this was interpreted as indicating an increased enzymatic activity;[48] it is very likely that these chemical findings merely reflect the advancing tumor decomposition and a consequent liberation of peptidases from the disintegrated tumor cells.

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[37] H. A. Colwell: Arch. Middlesex Hosp. *15*: 96 (1909).

[38] G. Mori: Acta dermat. *33*: 20 (1939).

[39] J. S. Fruton, G. W. Irving, Jr. & M. Bergmann: J. Biol. Chem. *132*: 465 (1940).

[40] H. von Euler, B. Skarżyński & B. Högberg: Ztschr. f. Krebsforsch. *50*: 230 (1940).

[41] M. E. Maver, J. M. Johnson & J. W. Thompson: J. Nat. Cancer Inst. *1*: 675 (1941).

The phenomenon of tumor autolysis is apparently related to proteolytic enzymes, but a complex nature of its mechanism must be assumed, since autolysis involves not only a far-reaching hydrolytic cleavage of proteins and polypeptides to amino acids, but also oxidation, reduction, deamination, and decarboxylation of the amino acids. Moreover, nucleoproteins and nucleic acids are included in this process of disintegration. An intensive autolytic activity of cancer tissue, as expressed by an increase of incoagulable nitrogen, has been reported in older studies.[49, 50] Some authors investigated the possibility as to whether an increased autolysis of normal organs may result from the admixture of neoplastic tissue, and they actually obtained results confirming such a phenomenon. The term of heterolysis has been

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42. O. Neubauer & H. Fischer: Deutsches Arch. f. klin. Med. *97*: 499 (1910).
43. H. Oppenheimer: Deutsches Arch. f. klin. Med. *101*: 294 (1911).
44. I. W. Hall & G. S. Williamson: Lancet *1*: 731 (1911).
45. H. Pechstein: Berlin. klin. Wchnschr. *48*: 375 (1911).
46. L. Ehrenberg: Berlin. klin. Wchnschr. *48*: 704 (1911).
47. H. Ley: Berlin. klin. Wchnschr. *48*: 119 (1911).
48. E. Zunz: Libro de Oro, Buenos Aires, p. 1520 (1935).
49. E. Petry: Hofmeisters Beitr. *2*: 94 (1902).
50. Ch. P. Emerson: Deutsches Arch. f. klin. Med. *72*: 413 (1902).

proposed for the property of neoplastic tissue to intensify the autolysis of normal tissue, and it was believed that this quality may be responsible for the development of cachexia in neoplasia.[51, 52] Yet no uniform opinion prevailed regarding this problem; one worker corroborated the earlier findings and observed an increased autolytic activity in apparently healthy areas of livers affected with metastases,[53] but other investigators were not able to reproduce these findings.[54, 55] In the light of recent work which showed that proteinases isolated from normal as well as from neoplastic tissue digested heterogeneous proteins,[21] the question of heterolysis seems somewhat to have lost its importance. Extensive studies on the autolysis of normal and neoplastic tissues yielded results which indicated that in these experiments an equilibrium is eventually reached between the protein decomposition and the resynthesis of protein split products (plastein formation); it was disclosed that variations of the pH markedly influenced this equilibrium.[56] One will not go amiss by correlating these observations with the illuminating results of the aforementioned studies on factors which determine proteolysis and protein synthesis.[27–29] Only one study has been devoted to autolysis in connection with chemical carcinogens in which, after being painted with benzpyrene, an increased autolytic activity of rabbit skin was observed. This change of an enzymic activity occurred prior to the development of malignancy, and it is noteworthy that benzpyrene, when added to tissue in vitro, inhibited the autolysis.[57] Mention should not be omitted of the fact that

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[51] C. Neuberg: Berlin. klin. Wchnschr. *42*: 118 (1905).
[52] F. Blumenthal: Berlin. klin. Wchnschr. *42*: 188 (1905).
[53] S. Yoshimoto: Biochem. Ztschr. *22*: 299 (1909).
[54] L. Kepinow: Ztschr. f. Krebsforsch. *7*: 517 (1909).
[55] L. Hess & P. Saxl: Wien. klin. Wchnschr. *21*: 1183 (1908).
[56] F. de Bruyne: J. d. physiol. et path. gén. *22*: 815; 849; 881 (1924).
[57] P. Rondoni & W. Beltrami: Enzymologia *3*: 252 (1937).

the problem of autolysis may also possess some practical significance, inasmuch as several measures proposed for treatment of cancer have been reported to act as a stimulation of autolysis, and, in this manner, to cause an intensified decomposition and perhaps a regression of tumors. Such opinions have been expressed in connection with heavy metal therapy (cf. chapter I), and experimental evidence was given for a similar action of radioactive substances. Radium administration in vivo activated autolytic tumor enzymes,[58] and addition of radium emanation in vitro increased the autolysis of human carcinomas and sarcomas to a higher degree than that of normal organs.[59] By investigating human breast cancers which, following roentgen radiation, had been removed surgically, it was ascertained that doses up to 2500 r increased the autolysis, whereas higher radiation amounts depressed the autolytic process.[60]

Serum proteinases. A number of proteolytic processes which have been believed to exhibit characteristic changes in the serum of tumor hosts will be discussed in chapter X, since these phenomena, which include the "protective ferments" of Abderhalden, and the fibrinolytic enzymes of Fuchs, have been studied mainly in connection with diagnostic purposes. Although it is conceivable that disintegration of tumor tissue leading to a flooding of the circulation with tumor proteins may cause an increase of serum proteinases in neoplasia, such an assumption is not supported by the results of investigations, according to which the titer of serum proteinases of rabbits is not changed following parenteral protein administration.[61] Actually, one investigator found a marked diminution of the proteolytic activity in eighty per cent of the sera of cancer patients, and it was inferred from these findings that a tendency to increased protein synthesis and to decreased protein cleavage may be associated with neoplasia.[62] Some support for such a theory may be seen in a later study, according to which rats with transplanted carcinomas or sarcomas showed diminished proteinases in their blood, and a similar change took place during pregnancy of normal rats. Removal of the tumors, or termination of the pregnancy, was followed by a return of the enzymatic activity to the normal; no change of the polypeptidase activity was found in the blood of tumor-bearing animals.[63] It is likely that no particular significance can be attributed to the finding of a normally lacking peptidase, able to split glycyl-tryptophane, in the sera of thirty-two out of thirty-six cancer patients tested, since this enzyme was detected also in sera of cachectic patients free of malignancy.[64] As mentioned above, because of investigations which claimed a presence of d-amino acids in the protein molecule of malignant tumors (Kögl, cf. chapter II), enzymatic studies have been initiated and devoted to a possible detection of polypeptidases able to split peptides with such components. Positive findings of such nature have been reported, inasmuch as sera of cancer patients were claimed to split eighty to one hundred per cent of dl-glutaminyl-glycine, or of dl-leucyl-glycine, whereas in sera of normal persons, the fission never exceeded fifty per cent, and therefore the presence of stereospecific peptidases was assumed for the sera of tumor patients.[65] In a further elaboration of these findings, it was assumed that these enzymes might be directly connected with the process of tumor development, since administration of racemic peptides inhibited the tumor appearance in mice treated with benzpyrene; also a clinical treatment with such peptides seemed to result in some improvement of tumor patients.[66] However, few studies confirmed the increased activity of d-peptidases in neoplasia[67] or gave evidence for a stronger cleavage of racemic peptides in sera of tumor patients than in those of tumor-free persons.[68] Most investigators obtained divergent results, inasmuch as neither sera of cancer patients, nor of normal individuals, brought about a fission of racemic peptides exceeding fifty per cent;[69] a higher enzymatic activity was found only in a small percentage of sera of tumor patients,[70-73] or in sera of rabbits with Brown-Pearce carcinoma, and of rats with Jensen sarcoma.[74] Likewise, recent work has demonstrated that the activity of splitting d-dipeptides is by no means specific for neoplasia, since an increase of this enzymatic process was found only in one out of nineteen tested sera of tumor patients, and was very rarely detected in sera of rats bearing Jensen sarcoma, whereas sera of older tumor-free rats frequently split more than fifty per cent of dl-leucylglycine.[75]

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[58] C. Neuberg: Ztschr. f. Krebsforsch. *2*: 171 (1904).

[59] S. Löwenthal & E. Edelstein: Biochem. Ztschr. *14*: 484 (1908).

[60] A. Faragó: Strahlentherapie *55*: 481 (1936).

[61] H. Kleinmann & G. Scharr: Biochem. Ztschr. *252*: 343 (1932).

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[62] E. Herzfeld & R. Klinger: Deutsche med. Wchnschr. *44*: 118 (1918).

[63] L. Weil & M. A. Russell: J. Biol. Chem. *126*: 245 (1938).

[64] R. Schaefer: Deutsches Arch. f. klin. Med. *161*: 313 (1928):

[65] E. Waldschmidt-Leitz & K. Mayer: Ztschr. f. physiol. Chem. *262*: IV (1939); *263*: I (1940).

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[66] E. Waldschmidt-Leitz & R. Hatschek: Ztschr. f. physiol. Chem. *264*: 196 (1940).

[67] E. Waldschmidt-Leitz, R. Hatschek & R. Hausmann: Ztschr. f. physiol. Chem. *267*: 79 (1940).

[68] H. Herken & H. Erxleben: Ztschr. f. physiol. Chem. *264*: 251 (1940).

[69] S. Ura: Gann *35*: 29 (1941).

[70] H. Bayerle & F. H. Podloucky: Ztschr. f. Krebsforsch. *50*: 220 (1940); Ztschr. f. physiol. Chem. *264*: 189 (1940).

[71] H. v. Euler, L. Ahlström, B. Skarżyński & B. Högberg: Ztschr. f. Krebsforsch. *50*: 552 (1940).

[72] H. v. Euler, L. Ahlström, B. Högberg & A. M. Lilja: Ztschr. f. Krebsforsch. *51*: 248 (1941).

[73] H. v. Euler & B. Skarżyński: Ztschr. f. physiol. Chem. *265*: 133 (1940).

[74] E. Abderhalden & R. Abderhalden: Ztschr. f. physiol. Chem. *265*: 253 (1940).

Anti-proteinases. Factors which inhibit the proteolytic activity have been claimed to be present in neoplastic tissue,[76] but the value of these findings seem rather doubtful because they were obtained prior to the development of precise knowledge concerning kinetics of enzymatic processes. A great amount of work has dealt with the antitryptic property of serum from the time it was reported that sera of tumor hosts inhibit the digestive action of trypsin to a greater extent than do sera of tumor-free persons.[77] Numerous attempts have been made to utilize this phenomenon for diagnostic purposes,[78-89] but the efficiency of this test, for the most part, failed to satisfy practical requirements. As to the mechanism of this trypsin inactivation, opinions were divided as to whether a specific substance in the sera causes this phenomenon, or whether changes in the serum composition are responsible.[78, 90–92] Investigations of more recent date appear to have decided for the second alternative, inasmuch as a shift of the hydrogen-ion concentration to the alkaline side,[93] or an increase of globulins,[78, 94] were reported to increase the antitryptic activity of sera; it may be recalled that such changes have been described as occurring frequently in neoplasia, although they represent merely secondary consequences of the tumor process (cf. chapters II and III).

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[75] M. E. Maver, J. M. Johnson & J. W. Thompson: J. Nat. Cancer Inst. *1*: 835 (1941).

[76] F. Fazio: abstr. Zentralbl. f. Biochemie *16*: 60 (1913/14).

[77] L. Brieger & J. Trebing: Berlin. klin. Wchnschr. *45*: 1041; 1349; 2260 (1908).

[78] von Bergmann & K. Bamberg: Berlin. klin. Wchnschr. *45*: 1396 (1908).

[79] von Bergmann & K. Meyer: Berlin. klin. Wchnschr. *45*: 1673 (1908).

[80] W. Schultz & R. Chiarolanza: Deutsche med. Wchnschr. *34*: 1300 (1908).

[81] E. Herzfeld: Berlin. klin. Wchnschr. *45*: 2182 (1908).

[82] O. Orszag & A. Barcza: Orvosi hetilap No. 34 (1909).

[83] F. Brenner: Deutsche med. Wchnschr. *35*: 390 (1909).

[84] Weinberg & U. Mello: Compt. rend. Soc. de biol. *67*: 434; 441 (1909).

[85] A. Torday: Budapesti orvosi ujsag No. 35 (1909).

[86] R. Schorlemmer & Selter: Ztschr. f. klin. Med. *69*: 153 (1910).

[87] A. Vecchi: Riforma med. *26*: 1156; 1188 (1910); abstr. Wien. klin. Wchnschr. *24*: 216 (1911).

[88] M. A. Dychno: abstr. Wien. klin. Wchnschr. *25*: 762 (1912).

[89] H. Guthmann & L. Hess: Arch. f. Gynäk. *131*: 462 (1928).

The urinary excretion of proteolytic enzymes has been studied in connection with the possibility of deriving information from such data as to the presence of gastric neoplasms; in this pathology a diminution of pepsin excreted in the urine has been observed, but the findings were neither regular nor uniform.[95–99] Obviously they depended upon the localization of the tumor, that is, whether or not the pepsin-producing glands of the stomach had been destroyed by the neoplasms. Here reference is made to a study in which low pepsin values have been determined in stomachs affected with cancer.[100]

Among enzymes of intermediate protein metabolism the *deaminases* have been included in the investigation of tumor enzymology and, as will be detailed in chapter VI, the ammonia formation of tumor slices in vitro, which obviously is due to an oxidative deamination of amino acids and of

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90. A. Braunstein: Deutsche med. Wchnschr. *35*: 573 (1909); Berlin. klin. Wchnschr. *47*: 478 (1910).
91. K. Meyer: Berlin. klin. Wchnschr. *46*: 1890 (1909).
92. O. Schwarz: Berlin. klin. Wchnschr. *46*: 2139 (1909).
93. F. Chrometzka: Ztschr. f. d. ges. exper. Med. *80*: 395; 408; 420; 439 (1932).
94. W. Raab: Klin. Wchnschr. *14*: 1633 (1935).
95. A. Ellinger & H. Scholz: Deutsches Arch. f. klin. Med. *99*: 221 (1910).
96. R. Bieling: Deutsches Arch. f. klin. Med. *102*: 507 (1911).
97. E. Fuld: Berlin. klin. Wchnschr. *45*: 1417 (1908); Zentralbl. f. exper. Path. *10*: 249 (1912).
98. S. Kosawa: abstr. Zentralbl. f. Biochemie *13*: 613 (1912).
99. K. Takeda: Deutsche med. Wchnschr. *36*: 1807 (1910).
100. C. R. Mullins & C. A. Flood: J. Clin. Investigation *14*: 793 (1935).

amino purines, has been found to be inversely proportional to the sugar content of the medium employed.[101] In carcinomas and sarcomas of rats a decrease of the deaminase activity has been found to accompany the aging of the tumors.[102] Another specific enzyme, viz., the transaminase, which converts l-glutamic acid and oxalacetic acid into α-ketoglutaric acid and aspartic acid, has been found diminished in Jensen rat sarcoma,[103] and other investigators observed a decreased transaminase activity in various mouse tumors and also in fetal tissue, as compared to adult tissues. It was suggested that this phenomenon, common to neoplastic and embryonic tissues, indicates an inverse ratio between transaminase activity and growth rate; that is, rapid growth may be associated with a decreased conversion of glutamic acid because this amino acid is required for the synthesis of complete protein molecules.[104] Additional evidence for an impairment of the transaminase activity in neoplastic tissue was furnished by an investigation, in which the activity of this enzyme was determined in livers of rats fed butter yellow. As compared with normal rat liver, the hepatic tissue of animals receiving the carcinogen showed a progressive decrease of the transaminase activity, and approximately one-third of the normal activity was found in the hepatomas eventually arising.[105]

Arginase. This enzyme which, in a specific manner, performs a hydrolytic cleavage of the amino acid arginine into ornithine and urea, has been investigated extensively in connection with tumor pathology, but with controversial results, a clarification of which will be attempted in the following outline. One of the first statements established the presence of arginase in metastatic liver cancer,[106] but failed to assess the quantitative relations. An elaboration of this problem was carried out in later studies, according to which a high arginase activity prevailed in all mammalian tumors tested.[107] This finding was supplemented by the observation that the arginase content of liver and spleen decreased rapidly following tumor transplantation to the animals and, although a similar phenomenon occurred in tuberculous animals, the arginase decrease in the organs of these animals required a much longer time.[108] Since an arginase increase has been found in embryonic tissue, in granulations, and in polyps, the authors regarded the high arginase activity of neoplasms as connected with their increased growth rate, and a direct correlation between mitotic activity and arginase was assumed.[109] Enzymes isolated from necrotic tumor parts or from non-necrotic areas respectively, were reported to exhibit a different reaction to the addition of reducing substances, such as cysteine or ascorbic acid, inasmuch as the arginase of tumor necrosis was more noticeably stimulated by these substances than the enzyme contained in intact tumor parts. This was regarded as being due to the fact that necrotic parts originally contained less reducing substances (cf. chapter III) and, therefore, the addition of such substances entails a more pronounced effect.[109] A difference between mammalian and fowl tumors was claimed since low arginase values were determined for Rous sarcoma,[107] but other workers contested this finding and reported an approximately equal arginase content of mouse carcinoma, mouse sarcoma, and fowl sarcoma.[110] As to other species, one worker observed a complete absence of arginase in one melanosarcoma of a horse.[18] The observation has been made that normal liver arginase is regularly inhibited by sulfhydryl compounds (cysteine, glutathione), whereas tumor arginase and the enzyme prepared from liver of tumor-bearing animals are frequently, but not always, activated by these substances.[111] As to the influence of the tumor process on other organs, it has been mentioned that a decrease of the enzyme was observed in liver and spleen after tumor inoculation,[108] and the low arginase content in the liver of tumor-bearing animals has been confirmed.[112] In addition, another remote effect of the tumor process concerned the muscles of tumor-bearing animals, which, in contrast to the absence of the enzyme in normal muscles, were reported to exhibit an arginase activity. A similar effect was found to follow the injection of tumor filtrates into normal animals,[110] but experiments to activate, with tumor filtrates, in vitro, arginase prepared from normal or neoplastic tissues yielded negative results.[113]

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[101] O. Warburg, K. Posener & E. Negelein: Biochem. Ztschr. *152*: 309 (1924).

[102] E. Waldschmidt-Leitz & E. McDonald: Ztschr. f. physiol. Chem. *219*: 115 (1933).

[103] H. v. Euler, H. Hellström, G. Günther, L. Elliot & S. Elliot: Ztschr. f. physiol. Chem. *259*: 201 (1938).

[104] P. P. Cohen & G. L. Hekhuis: Cancer Research *1*: 620 (1941).

[105] P. P. Cohen, G. L. Hekhuis & E. K. Sober: Cancer Research *2*: 405 (1942).

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[106] B. Fuchs: Ztschr. f. physiol. Chem. *114*: 101 (1921).

[107] S. Edlbacher & K. W. Merz: Ztschr. f. physiol. Chem. *171*: 252 (1927).

[108] H. Fujiwara: Ztschr. f. physiol. Chem. *185*: 1 (1929).

[109] S. Edlbacher & F. Koller: Ztschr. f. physiol. Chem. *227*: 99 (1934).

Although the reports enumerated seem to give definite evidence for an increased arginase content in malignant tumors and a changed distribution of the enzyme in the organs of tumor hosts, the results of later investigations failed, to a large extent, to corroborate these findings. The arginase activity of various transplanted animal tumors (rabbit sarcoma, rabbit carcinoma, fowl sarcoma, mouse carcinoma) was found to be relatively low, but corresponded approximately to the values obtained for normal liver, when correlated to dry tissue substance, i.e., when the increased water content of tumors was taken into consideration. No relation between tumor necrosis and arginase content was observed. Likewise there was no different behavior of normal and tumor arginase, as to the activating effect of manganese salts, nor were characteristic changes ascertained as to the arginase activity in the body of tumor hosts with the exception of a slight increase of the muscle arginase in mice bearing large tumors.[114] The author of the previous communications, however, reported in contrast to the results of animal experimentation, that the arginase activity was increased in human gastric carcinomas and he noted a parallelism between the growth rate of these tumors and their arginase content.[115] The discrepancies exhibited by the reports enumerated have been partly clarified and corrected by recent investigations in which improved technical methods permitted a more precise evaluation of the results obtained. Furthermore, the authors of these studies stressed the necessity of comparing the enzyme activity of tumor tissues with that of the respective normal parent tissue. They used for such purposes transplanted hepatic tumors of mice and rats, spontaneous mammary cancer of mice, and lymphomas of mice. In all these instances the tumor arginase was studied parallel to the enzyme activity of the normal organs from which the tumors originated, and also embryonic liver, lactating, and hyperplastic mammary tissues were included in the investigation. In this manner it was ascertained that the arginase activity of hepatic tumors and of embryonic liver is much less than that of normal hepatic tissue, but spontaneous mammary mouse tumors exhibited a markedly greater activity than the normal control tissue, and mouse lymphomas showed a slightly greater enzyme activity than normal lymph nodes. Determinations of the enzyme activity in a number of miscellaneous tumors yielded generally low values, approximately of the same order of magnitude as in hepatic tumors. The pH optimum of arginase and the effect of manganese addition were found to be identical for liver and tumor arginase, and a decrease of the liver arginase of tumor-bearing animals did not occur except in some instances of very rapidly growing hepatic tumors in rats.[116] Hence it does not seem possible to ascribe to neoplasms in general an increased arginase activity, and organs of tumor hosts appear to be affected as to their enzymatic activity only when the tumor process advances very rapidly. Transplanted tumors of the latter type may have been used in some of the previously discussed studies, and this fact, together with deviations in the methods employed, may account for the conflicting results noted in former works.

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[110] G. Klein & W. Ziese: Ztschr. f. Krebsforsch. *37*: 323 (1932).
[111] G. Klein & W. Ziese: Ztschr. f. physiol. Chem. *213*: 201 (1932).
[112] L. Weil: J. Biol. Chem. *110*: 201 (1935).
[113] G. Klein & W. Ziese: Ztschr. f. physiol. Chem. *213*: 217 (1932).

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[114] M. Kaiju: J. Biochem. 27: 35; 387 (1938).

[115] M. Kaiju: J. Biochem. 29: 81 (1939).

Divergent findings have also been obtained for the blood arginase of tumor hosts: on the one hand an increase was found in eight out of thirty-two cases of cancer patients tested,[117] whereas a marked diminution was noted in the blood of tumor-bearing rats.[118] Since, however, blood normally contains only very small amounts of arginase [107] these changes cannot be considered to be very significant.

In conjunction with proteolytic enzymes, brief mention is made of the enzymes of blood coagulation, viz., thrombokinase and thrombin. Only a few studies have been devoted to this problem in connection with malignant tumors and the following controversial statements have been made. In advanced human neoplasia an accelerated blood coagulation was found;[119] but a comparison of patients with gastric cancer or with ulcer indicated a retardation of the coagulation in the first condition,[120] and tumor-bearing rabbits also were reported to exhibit a delayed blood coagulation.[121] These divergencies may be due to the complex mechanism of blood coagulation which, as is well known, depends not only upon enzymatic factors but also upon the presence of calcium, the number of thrombocytes and the fibrinogen concentration, all of which may be secondarily influenced, to a varying extent, by the tumor process. A characteristic difference has been claimed to exist between blood corpuscles of tumor hosts and tumor-free individuals as to their influence on blood coagulation; that is, the coagulation of heparinized plasma was reported to be stimulated by extracts of leucocytes of tumor-free animals or humans but was not stimulated by extracts of erythrocytes, whereas the erythrocyte but not the leucocyte extract of animals or humans afflicted with neoplasia brought about a similar effect.[122, 123] This phenomenon was thought to be useful for diagnostic purposes, yet, as will be shown in chapter X, re-examinations failed to supply a confirmation.

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[116] J. P. Greenstein, W. V. Jenrette, G. B. Mider & J. White: J. Nat. Cancer Inst. *1*: 687 (1941); J. Biol. Chem. *137*: 795 (1941).

[117] A. Haddow: Lancet *1*: 1021 (1931).

[118] L. Weil & M. A. Russell: J. Biol. Chem. *106*: 505 (1934).

[119] N. Louros & Gaessler: Ztschr. f. Krebsforsch. *28*: 191 (1929).

Therapeutic use of proteinases. Measures of this kind are apparently based on the rather primitive idea that the destruction of neoplastic tissue may be accomplished by means of proteolysis. Indirect support for such an assumption has been seen in the fact that malignant tumors occur only very rarely in the small intestines, and this circumstance was believed to be due to the abundant presence of proteolytic enzymes in this particular part of the intestines.[123] An experimental foundation for therapeutic experiments was furnished by findings which demonstrated neoplastic tissue to be more resistant against the action of pepsin than normal tissues but, in comparison with the latter, to be more sensitive against tryptic enzymes.[8, 124–127] This behavior of tumor tissue was verified more recently in Ehrlich mouse carcinoma and Brown-Pearce rabbit carcinoma by using modern methods. It is noteworthy that no significant differences as to the tryptic or peptic digestibility were observed in organs of tumor-free or tumor-bearing animals;[128] the plant proteinase papain was found to cause a still greater cleavage of mouse tumors than that caused by trypsin, and the sensitivity of organs of tumor-bearing mice against papain appeared greater than that of organs of tumor-free animals.[129] Based on an entirely different aspect, namely on the alleged presence of d-amino acids in the molecule of tumor protein, an attempt has been made to demonstrate a decreased digestibility of tumor protein in the animal body. This assumption was believed to have been proved by means of measuring the excretion of glutamic acid in peptide linkage after feeding boiled Brown-Pearce carcinomas to dogs.[130] Yet, in contrast to this claim, another worker failed to notice any difference in the proteolytic digestibility of normal protein and of protein derived from human neoplasms or from Brown-Pearce carcinomas.[131]

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[120] Bock & Rausche; quot. H. Auler: Neue deutsche Klinik *5*: Erg.-B., 182 (1937).

[121] C. M. Van Allen: Proc. Soc. Exper. Biol. & Med. *21*: 466 (1924).

[122] P. Mendeleéff: Cancer, Bruxelles *11*: 135 (1934); Compt. rend. Soc. de biol. *119*: 1190 (1935).

[123] J. A. Shaw-Mackenzie: The nature and treatment of cancer, Baillière, Tindall & Cox. London 1905.

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124. C. Neuberg: Berlin. klin. Wchnschr. *42*: 1189 (1905).

125. P. Bergell & T. Dorpinghaus: Deutsche med. Wchnschr. *31*: 1426 (1905).

126. C. Lewin: Ergebn. d. inn. Med. u. Kinderh. *2*: 168 (1908); Die bösartigen Geschwülste, Leipzig 1909; abstr. Wien. klin. Wchnschr. *24*: 674 (1911).

127. K. Hübschmann: Dermat. Wchnschr. *73*: 1145 (1921).

128. A. Langer: Tumori n.s. *10*: 247 (1936); *11*: 412 (1937).

129. A. Langer: Tumori n.s. *10*: 430 (1936).

130. F. Kögl & H. Erxleben: Ztschr. f. physiol. Chem. *264*: 108 (1940).

131. H. Bayer: Biochem. Ztschr. *303*: 251 (1939); *305*: 227 (1940).

Returning to endeavors to utilize proteinases for cancer therapy, it must be conceded that some reports are available, according to which trypsin administration resulted in favorable effects when used in skin cancer [132] and other types of human neoplasia,[133, 134] or in animal tumors.[135] Yet the prevailing majority of trypsin therapy in animals [136, cf. 134] or in clinical cases [cf. 137] led to entirely negative results, and in 1912 one author evaluated a great number of reports on this therapy in the following manner: ". . . this therapy has not proved to be an essential contribution to the treatment of cancer. The successes announced are of highly problematic nature and by no means justify any hopes. Most commonly the favorable results of the discoverers remain the only ones . . :" [138] More recent precise investigations on the action of trypsin on human or animal tumors (Brown-Pearce rabbit carcinoma) [98, 139, 140] cannot be regarded as warranting a more optimistic view. It is true that in these studies the increased sensitivity of cancer cells against trypsin in vitro has been confirmed; a 0.6 per cent trypsin solution caused complete destruction of tumor cells within twenty-four hours, a finding which was not obtained with normal tissues, with the exception of testis, thyroid and fetal liver. Yet the possibility of a therapeutic utilization of this reaction of tumor tissue has been denied on account of theoretical considerations (intravenous injection of trypsin immediately results in a very high dilution of the enzyme) and, particularly, because of the observation that tumor hosts tolerate trypsin administration very poorly, severe toxic effects of this treatment have been encountered in cancer patients.[93] Favorable experiences were reported as resulting from the injection of one to two per cent pepsin solutions into prostatic carcinomas.[141] Yet the number of cases treated was far too small to permit an evalution of this procedure. Negative results were obtained with the plant proteinase papain, inasmuch as this enzyme failed to influence the growth of Ehrlich mouse carcinoma.[20] Some proteinases of bacterial origin have also been tested; according to an older report, rat sarcomas were brought to regression by means of intratumoral injections of pyocyanase,[142] and a successful treatment of rat carcinomas with proteolytic enzymes of bacillus histolyticus and bacillus sporogenes was announced.[143] More recently far reaching claims have been advanced as to the curative value for cancer of enzyme preparations derived from bacillus histolyticus by special methods. Animal experiments, as well as clinical experiences, were enumerated which allegedly proved great effectiveness of this product called "ensol." [144] Yet a number of investigations of other workers performed with various animal tumors failed to reveal any curative effect of ensol,[145–148] nor did experiments with neoplastic tissue cultures demonstrate any growth inhibition as resulting from the addition of ensol [149] and, moreover, several cases of death

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[132] W. J. Morton: Prag. med. Wchnschr. 1907: Nos. 17–19; abstr. Ztschr. f. Krebsforsch. *6*: 633 (1908).

[133] A. Sticker & E. Falk: Berlin. klin. Wchnschr. *47*: 1049 (1910).

[134] G. Fichera: Chimioterapia del cancro, Ulrico Hoepli, Milano 1934.

[135] H. Tamura: Tr. Jap. Path. Soc. *17*: 538 (1929).

[136] S. Rushmore: J. Med. Res. *21*: 591 (1909).

[137] Prampolini; Dilibert-Herbin; quot. G. Fichera l.c. 134.

[138] C. Lewin: Ergebn. d. inn. Med. u. Kinderh. *8*: 499 (1912).

[139] W. Raab & J. Breuer: Ztschr. f. d. ges. exper. Med. *97*: 415 (1935).

[140] W. Raab: Ztschr. f. d. ges. exper. Med. *97*: 588 (1935).

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141. Payr: Zentralbl. f. Chir. *54*: 1858 (1927).
142. P. T. Uhlenhuth: abstr. Ztschr. f. Krebsforsch. *9*: 93 (1910).
143. J. C. Torrey & M. C. Kahn: J. Cancer Res. *11*: 334 (1927).
144. H. C. Connel: Canad. M. A. J. *33*: 364 (1935); Bull. Hendry-Connel Res. Found. *1*: 24 (1936).
145. W. E. Gye: Brit. M. J. *2*: 760 (1935).
146. W. T. Pommerenke: J. A. M. A. *106*: 1654 (1936).
147. H. P. Rusch & W. S. Preston: Proc. Soc. Exper. Biol. & Med. *34*: 581 (1936).
148. W. Moppett & N. E. Goldsworth: M. J. Australia *2*: 754 (1936).
149. A. Goldfeder: Am. J. Cancer *33*: 560 (1938).

occurring in patients treated with ensol resulted in the rejection of the continuation of this therapy, a real value of which has not been observed by critical investigators.[150]

2. *Nucleinases.*

Particular interest is to be assigned to studies of these enzymes which act on substrates which are found predominantly as constituents of cell nuclei because of their possible connection with changes in the nuclear chemistry and in the mitotic activity of malignant cells. The presence of nucleinases in neoplastic tissues was demonstrated a long time ago.[151] Later work did not disclose any qualitative difference between the tumor enzymes and those contained in normal organs so far as the effects on the enzymatic activity of prussic acid, cysteine, glutathione and ascorbic acid are concerned;[152, 153] anaerobic conditions were shown to increase the activity of tumor nucleases.[153] It was demonstrated that extracts of Rous sarcomas of fowls contained an enzyme capable of splitting purine bases from yeast nucleic acids[154] and, likewise a considerable polynucleotidase activity was observed in fowl tumor extracts by means of using nucleic acid from yeast as substrate. As compared with normal organs, the activity of tumor extracts ranged between that of the kidney and of the spleen, appreciably exceeding that of muscle; it was found to be approximately parallel to the tumor-producing potency of the extracts.[155] As to enzymes attacking cleavage products of nucleic acids, viz., the purine bases, an older study used an indirect method, that is, determinations of free purine bases were performed in tumor tissue, and the results obtained made the presence both of guanase and of xanthine dehydrogenase and the absence of adenase probable,[156] relations which would correspond to those found in normal parenchymatous organs. Yet direct enzymatic investigations have disclosed the absence of xanthine dehydrogenase in tumors,[157] and this was particularly impressively shown in the case of metastatic liver cancer, where the normal hepatic tissue contained abundant quantities of the enzyme.[158] Exact studies recently performed established that the xanthine dehydrogenase of transplanted hepatic rat tumors was only half of that of normal rat liver and, also, that fetal or regenerating liver exhibited a lower enzymatic activity than normal adult liver.[159] Yet this depression of the xanthine dehydrogenase activity appears to be by no means a general property of neoplastic tissue, since the authors of the previous study,[159] by extending their work to various mouse tumors, found that some hepatic tumors of mice (dilute brown strain) exhibited the same activity as normal mouse liver, whereas hepatic tumors of the C 3H strain showed a much smaller activity. Greater enzymatic activities as compared with the respective normal tissues were observed in spontaneous mammary tumors and, especially, in lymphomas of mice.[160]

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[150] ——————————: J. A. M. A. *110*: 1183 (1938); *116*: 2774 (1941).

[151] E. H. Goodman: J. Exper. Med. *15*: 477 (1912).

[152] S. Edlbacher & W. Kutscher: Ztschr. f. physiol. Chem. *207*: 1 (1932).

[153] M. E. Maver & C. Voegtlin: Am. J. Cancer *25*: 780 (1935).

[154] E. Somekawa: Scientif. Pap. Inst. Phys. & Chem. Research *18*: 23 (1932); abstr. Ronas Ber. *67*: 51 (1933).

[155] D. A. MacFadyen: Am. J. Cancer *22*: 597 (1934); J. Exper. Med. *60*: 361 (1934).

A new enzymatic factor has recently been demonstrated which brings about a depolymerization of thymonucleic acid,

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156. T. Saiki: J. Biol. Chem. *7*: 23 (1909).
157. H. G. Wells: Proc. Am. Soc. Biol. Chem., 6th meet., p. 10 (1911).
158. H. G. Wells & E. R. Long: Ztschr. f. Krebsforsch. *12*: 598 (1919).
159. J. P. Greenstein, W. V. Jenrette & J. White: J. Nat. Cancer Inst. *2*: 17 (1941).
160. J. P. Greenstein, W. V. Jenrette, C. B. Mider & H. B. Andervont: J. Nat. Cancer Inst. *2*: 293 (1941).

as expressed in changes of the viscosity and of the streaming birefringence of the nucleic acid. This enzymatic action has been extensively studied and clearly differentiated from a similar effect caused by proteins and salts. The presence of this enzyme, called thymonucleodepolymerase, has been demonstrated in a number of normal organs and in the milk and serum of some animal species. Comparisons of this enzyme in normal and neoplastic tissues showed mammary mouse tumors to be more active than lactating or hyperplastic mammary tissue,[161] whereas some hepatic tumors were less active than normal or regenerating liver,[160, 161] and mouse lymphomas also showed a much weaker activity than the extremely active normal lymph nodes.[161] In conjunction with observations of the influence of protein and salts on the physical state of nucleic acids, the authors referred to an important bearing of the thymonucleodepolymerase on the formation of nucleoproteins in the cells, a problem on which further experimental work is expected.[161]

An increased nucleolytic activity has been reported for sera of tumor patients [162] but, since similar findings were obtained in tuberculous patients, it is plausible to regard this phenomenon merely as an expression of tissue disintegration and of a subsequent entrance of tissue enzymes into the blood stream. The guanase content of the spleen of tumor-bearing rats was found markedly increased, and some differences were also observed as to the activation of this enzyme when compared with normal organ enzymes.[163] Finally, thymonucleases have been shown to be present in the urines of tumor patients although similar findings were also reported

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161. J. P. Greenstein & W. V. Jenrette: J. Nat. Cancer Inst. *1*: 845 (1941).

162. B. Wolter: abstr. Zentralbl. f. Biochemie *16*: 271 (1913/14).

163. I. Rydh-Ehrensvärd & G. Schmidt: Ztschr. f. physiol. Chem. *227*: 177 (1934).

for other pathologies.[164] A certain connection perhaps exists between these enzymes and an interesting study reporting on the presence in urines of enzymes capable of splitting nucleoproteins; that is, great significance has been attributed to the observation that the urine of a diseased person fails to split nucleoproteins derived from the same organ which is pathologically affected in the patient. Technically, this phenomenon was found after mixing a urine portion with nucleoproteins derived from various organs and, following incubation of the mixture, acetic acid was added; a positive outcome, i.e., a pathology of a certain organ was believed to be recognizable when a precipitation occurred after the addition of acid, thus indicating the presence of undigested nucleoproteins.[165] Yet, as will be shown in chapter X, the practical experiences with this test, supposedly useful for establishing the location of a tumor, have not so far reached a stage which permits a definite evaluation..

3. *Lipases.*

The majority of investigators have arrived at the conclusion that lipases are present in malignant tumors,[166] but quantitative estimations reveal a comparatively low enzyme content. Thus, the magnitude of the lipatic activity of the Flexner-Jobling rat carcinoma corresponded approximately to that of muscle, a tissue with much lower enzyme activity than parenchymatous organs. The tumor-free organs of tumor hosts showed lipase values which did not differ from those of control animals.[167] Determinations of the lipatic activity of extracts of Ehrlich mouse carcinomas yielded values one-seventh of that of liver extracts and about equal to those of spleen extracts.[168] The lipase content of Jensen rat sarcomas was found to be approximately one-fiftieth of that of normal rat liver, i.e., identical with that of rat brain; in this instance also the organs of tumor-bearing rats were reported to show a decreased lipatic activity, and this finding was interpreted as a consequence of cachexia.[169] Uterine carcinomas were found devoid of lipase or lecithinase, a finding which corresponds to those obtained in normal uterine tissue.[170] To what extent reports of the complete absence of lipase in several human neoplasms [17, 171] have been due to the types of tumors tested or to inadequate methods cannot be decided. The esterase activity of Brown-Pearce rabbit carcinoma was reported to decrease on a course parallel to the advancing necrosis of the primary or of metastatic tumors; organs of tumor animals (liver, kidney, testis) exhibited only slight changes of the enzyme activity, or a preterminal reduction.[172] As far as the effect of carcinogens is concerned, only one report is available, according to which painting of rabbits with benzpyrene resulted in an increased lipase content of the areas treated, as compared with normal skin.[57]

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[164] A. Dmochowski: Compt. rend. Soc. de biol. *113*: 956 (1933).

[165] E. Freund: Wien. klin. Wchnschr. *46*: 1547 (1933)

[166] B. H. Buxton & P. Shaffer: J. Med. Res. *9*: 356 (1903); *13*: 543 (1905).

[167] K. G. Falk, H. M. Noyes & K. Sugiura: J. Biol. Chem. *59*: 183; 225 (1924).

The activity of the serum lipase has been found markedly lower in some cancer patients, and since similar findings were also obtained in advanced tuberculosis, it seems probable that this enzymatic alteration was dependent on cachexia.[173] This circumstance may explain conflicting reports which have been issued on this question. A decreased lipase

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168. P. Rona & A. Lasnitzki: Biochem. Ztschr. *152*: 504 (1924); *173*: 207 (1926).

169. S. Edlbacher & M. Neber: Ztschr. f. physiol. Chem. *233*: 265 (1935); Schweiz. med. Wchnschr. *65*: 731 (1935).

170. K. Nakahori: Jap. J. Obst. & Gynec. *17*: 419 (1934).

171. B. Brahn: Preuss. Akad. d. Wissensch. *34*: 680 (1910).

172. G. Mori: Acta dermat. *32*: 31 (1938).

173. J. Bauer: Wien. klin. Wchnschr. *25*: 1376 (1912).

activity has been observed in sera of a few tumor patients tested,[174] but other workers found normal values in patients afflicted with non-pancreatic tumors, whereas pancreatic carcinomas, as well as other pancreas pathologies, were accompanied by high values of the serum lipase.[175] A decrease of the serum lipase has been found in rats inoculated with Jensen sarcomas or treated with tar, whereas tumor-resistant animals did not show this change.[176] Inoculation of carcinomas into rats was found to be followed by a considerable drop of the blood lipase activity, but the values returned to the normal after regression of tumors,[177] and about eighty per cent of rabbits inoculated with Brown-Pearce carcinoma exhibited a diminished esterase activity in the serum.[172] Yet other workers failed to notice any alteration of the serum esterases in tarred animals,[178] or even reported an increase of the lipase activity in sera of rabbits painted with tar or benzpyrene.[179]

A qualitative disturbance of the lipatic enzymes in neoplasia has been observed, inasmuch as the atoxyl-resistant fraction of serum lipase was found to be increased in the majority of cancer patients.[180] Although, normally, resistance against atoxyl is characteristic for pancreatic lipase, the origin of atoxyl-resistant serum lipase in cancer patients must apparently be sought in the tumors themselves, since surgical tumor removal causes a disappearance of this enzyme. Actually, an increase of atoxyl-resistant lipase has been detected in extracts of various human and animal tumors [181] and, for this reason, it cannot be assumed that the occurrence of this enzyme in the sera of tumor patients was due to pancreas damage as has been observed in other pathologies.[182] As will be explained in chapter X, this enzymatic alteration has been utilized as a means of tumor-diagnosis, but its usefulness is impaired by the circumstances that, on the one hand, the atoxyl-resistant lipase frequently disappears from the serum in advanced stages of the tumor process, whereas on the other hand, it may also be encountered in the presence of increased quantities of newly formed epithelial elements, such as in regenerative processes or in pregnancy.[180]

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[174] H. Hangleiter & A. Reuter: Ztschr. f. d. ges. exper. Med. *107*: 355 (1940).

[175] T. A. Johnson & H. L. Bockus: Arch. Int. Med. *66*: 62 (1940).

[176] H. N. Green: Brit. J. Exper. Path. *15*: 1 (1934).

[177] E. E. Troescher & E. R. Narri: J. Biol. Chem. *132*: 553 (1939).

[178] B. Sure, M. C. Kik, K. S. Buchanan, H. S. Thatcher & A. DeGroat: Proc. Soc. Exper. Biol. & Med. *32*: 658 (1935); Biochem. J. *29*: 1508 (1935).

[179] W. Beltrami: Tumori n. s. *11*: 429 (1937).

[180] F. Bernhard: Ztschr. f. Krebsforsch. *38*: 450 (1932/33).

Activators of lipatic enzymes which are normally found in sera have been reported to be diminished in neoplasia.[183–185] Improvements in the clinical status of cancer patients, subjected to lead therapy, were accompanied by a return of the lipase-activating capacity of the serum to the normal; hence a utilization of these determinations for prognostic purposes was deemed feasible.[184, 185] In this connection it may be mentioned that lipase-activators have been isolated from mesenchymal tissues, and have been regarded as being associated with the phenomenon of carcinolysis [186] (cf. chapter X). In this manner this disturbance of a lipatic mechanism in neoplasia may be connected with the damaged mesenchymal function, which latter problem will be extensively discussed in chapter VIII.

Regarding therapeutic experiments, reference is made to a

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181. K. Köhler: Ztschr. f. Krebsforsch. *43*: 87 (1936).
182. C. H. Lasch: Arch. f. klin. Chir. *150*: 272 (1928).
183. J. A. Shaw-Mackenzie: J. Physiol. *42*: 1 (1911); *49*: 216 (1915).
184. W. C. M. Lewis: Internat. Conference Cancer, London, p. 437 (1928).
185. R. F. Corran & W. C. M. Lewis: Biochem. J. *22*: 451 (1928).
186. N. Waterman: Ztschr. f. Krebsforsch. *34*: 313; 327 (1931).

report of favorable clinical results achieved by treatment with a titanium-lipase preparation,[187] and to growth inhibitions or cures of rat tumors, following administration of a pancreas extract containing lipase.[188] Yet the scope and number of these studies is far too meager to permit any definite conclusions, and continued investigations may be considered desirable, in view of the close connection apparently existing between lipatic enzymes and carcinolysis.

4. *Phosphatases.*

These esterases may require particular attention in connection with tumor enzymology, since they participate in the processes of phosphorylation in the intermediary carbohydrate metabolism, and since characteristic alterations of the carbohydrate metabolism in neoplasia have been established, as will be described in chapter VI. Furthermore, phosphatases play an important part in the physiology and pathology of bone formation [189] and, actually, involvement of the skeleton in neoplasia has been found to result in definite alterations of the phosphatase distribution, as will be shown later. In one of the first investigations on the subject, a high content of glycerophosphatase [190] and also saccharophosphatase [191] has been established for carcinomatous tissue. Whereas the former enzyme is present in all epithelial organs and may therefore be inherent in carcinomas by virtue of their origin, the saccharophosphatase, an enzyme directed against a synthetic phosphoric ester, is normally found only in the pancreas and adrenals.[191] Nucleophosphatases

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187. R. Gardner: J. Trop. Med. & Hyg. *31*: 194 (1928); abstr. Cancer Review *4*: 578 (1929).

188. B. Ottenstein & S. Pastinszky: Ztschr. f. Krebsforsch. *42*: 1 (1935).

189. R. Robison: Biochem. J. *17*: 286 (1923).

190. E. Forrai: Biochem. Ztschr. *142*: 282 (1923).

191. E. Forrai: Biochem. Ztschr. *144*: 149 (1924); Ztschr. f. d. ges. exper. Med. *43*: 647 (1924).

have been investigated in various animal tumors (rat, mouse and fowl sarcomas); the mammalian tumors in particular exhibited a high enzymatic activity, as expressed in the cleavage of nucleic acids derived from animals or yeast,[192] and the muscles of tumor-bearing animals also showed an increased phosphatase activity.[192, 193] Yet, when the authors of the latter communications arrived at the conclusion, on the basis of these and previous findings relating to arginase, that tumor enzymology is characterized by a prevalence of hydrolytic enzymes and a simultaneous depression of enzymatic oxidation,[192] it must be remembered that, as previously shown, great differences exist in the enzymic activity of various types of tumors, and hence a general statement of this nature appears for the time being to be unfounded. Furthermore, it has been conceded that an increased phosphatase activity is probably common to all rapidly growing tissues,[192] and this view seems supported by comparative analyses of phosphatases in benign and in malignant tumors of the uterus, in which latter a much higher enzymatic activity has been determined.[194] Studies on the kinetics of tumor phosphatases showed a slight inhibiting effect on the enzyme of ascorbic acid.[153] It was reported that the degree of activation as measured by the effect of magnesium addition was very high in animal tumors (fifty-eight to one hundred per cent), whereas it was decreased in the kidneys of the animals (27.8 to 49.6 per cent as compared with normal values of 96.5 to 100 per cent).[195] An indirect approach made it probable that the body of tumor hosts possesses an increased phosphatase activity, inasmuch as after intravenous

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192. S. Edlbacher & W. Kutscher: Ztschr. f. physiol. Chem. *199*: 200 (1931).

193. J. Wienbeck: Ztschr. f. physiol. Chem. *219*: 164 (1933).

194. M. Morii & Y. Takabatake: Arb. a. d. III. Abt. d. Anat. Inst. Kyoto *3*: 46 (1932); abstr. Ztschr. f. Krebsforsch. *39*: Ref. 61 (1933).

195. F. Köhler: Ztschr. f. physiol. Chem. *223*: 98 (1934).

injection of sodium glycerophosphate cancer patients showed a much larger excretion of inorganic phosphorus, apparently a split product of the glycerine ester, than was observed from an analogous procedure in persons free of tumors; yet a diagnostic utilization of this phenomenon has not been considered as warranted.[196] Very valuable information has been derived from separate investigations of phosphatases which possessed an alkaline or an acid optimum reaction; an enzyme of the latter quality is regularly found in the prostate and, significantly, an increased content of "acid" phosphatase has also been ascertained in osteoplastic bone metastases of prostatic cancer, while normal values were found in osteolytic bone metastases.[197] With regard to the physiology of the "alkaline" phosphatase, histochemical methods proved the epithelia of small intestines, the endothelia and the osteoblasts to be very rich in this enzyme. Accordingly, conspicuous amounts of "alkaline" phosphatase have been found in malignant osteoblasts of a transmissible chicken sarcoma, and in osteogenic mouse tumors.[198] It was emphasized that this enzyme is physiologically important for bone formation and for processes of phosphorylation taking place in the intestines and kidneys, and it may be anticipated that the findings previously mentioned [197, 198] will prove of great value for the understanding of investigations concerned with the serum phosphatases in neoplasia, to be discussed in the next section. Detailed information was recently presented with regard to the values of acid and alkaline phosphatases in normal tissues of mice and rats as well as in a great many neoplasms of these animals. By comparing the relative activity of the enzymes, a predominance of the acid phosphatase

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196. E. Tantini & G. De Poli: Tumori n. s. *13*: 602 (1939).

197. E. B. Gutman, E. E. Sproul & A. B. Gutman: Am. J. Cancer *28*: 485 (1936).

198. E. A. Kabat & J. Furth: Am. J. Path. *17*: 303 (1941).

over the alkaline phosphatase was found in the liver, spleen, muscle, skin; in all transplanted mouse tumors tested and in mouse tumors induced by benzpyrene. An approximately equal activity of acid and alkaline phosphatases was determined for lung and bone marrow of mice; for spontaneous mammary tumors and a lymphoma of mice. The following tissues showed a lesser activity of acid phosphatase than of alkaline phosphatase: intestinal mucosa, kidney, Jensen rat sarcoma and transplanted hepatoma 31 of rats. It is significant that the normal tissues and the tumors of the two animal species exhibited marked differences as to their enzymatic activity. Mouse tumors (excepting lymphomas and spontaneous mammary tumors) showed an increased activity of acid phosphatase, as compared with normal mouse tissues, whereas the activity of the alkaline phosphatase was very slight or entirely absent. In rat tumors, on the other hand, a high activity of alkaline phosphatase was observed; this was especially pronounced in transplanted hepatic tumors when compared with normal rat liver. In general, the absolute values of the acid phosphatase were higher in normal and malignant tissues of rats than in normal and malignant tissues of mice.[199]

An increase of phosphatases has been reported for the blood of tumor patients and of tumor-bearing animals,[195, 200] although other workers observed a slight diminution of blood phosphatases in rats inoculated with Walker carcinomas.[178] Recent work, discussed previously,[199] failed to reveal any significant change in the phosphatase activity of sera of mice and rats with transplanted tumors, nor did the liver, kidney, skin and muscles of these animals exhibit deviations from the normal as to their phosphatase activity. Among fifty cancer patients tested (mostly gynecological cancers) a phosphatase increase was found in only two instances, in the absence of liver metastases.[201] In this connection, it must be borne in mind that phosphatase increases in serum are also encountered in non-malignant conditions, such as bone diseases and icterus;[202] yet in spite of these facts, and in contrast to the aforementioned findings, a recent study claimed the phosphatase activity of sera of tumor patients to be very regularly increased, although similar findings were also obtained in jaundice, tuberculosis and in stomach ulcers.[203] Divergent results have been recorded as to the effect of the addition of magnesium on blood phosphatases, differences which may have been due to variations in the technique and substrates employed; according to one study, hemolysates of erythrocytes of cancer patients are activated to a lesser degree than normal, and this alteration was particularly pronounced as to the β-glycerophosphatase.[204] Another investigator claimed a greater activating effect on serum phosphatases of tumor patients from magnesium, as compared with normal relations.[203] In agreement with the latter report, the phosphatases of cancer serum were found to be activated to a much higher degree than normal serum enzymes (averaging forty-two per cent as compared with 5.8 per cent), whereas calcium salts caused a stronger inhibition of the cancer serum phosphatase. The effect of sodium fluoride did not differ in sera of normal or neoplastic persons, and the inhibition due to mono-iodoacetate was so strong as to render any differentiation impossible.[205] In general, the phosphatase increase in the serum of cancer patients was recognized as dependent on the occurrence of liver metastases or of bone metastases;[206, 207] in the latter cases the changes occurred even prior to the clinical manifestations of the skeleton involvement[208] and, similarly, increased serum phosphatases observed in Boeck's sarcoid have been connected with bone lesions.[209] In particular, investigations which extended to 200 patients demonstrated serum phosphatases to be an indicator for bone involvement, since roentgenologic examinations proved that osteoplastic processes of malignant nature were accompanied by high serum phosphatase values, whereas low values were found in osteolytic processes. Osteoplastic processes, associated with low phosphatase values, were considered to exclude the presence of malignancy, while high enzyme activity in patients with osteolytic processes could indicate either hyperparathyroidism or malignant osteosarcoma. Accordingly, the great value of serum phosphatase determinations for differential diagnosis has been stressed, and their use has also been recommended for prognostic purposes (recurrence, formation of metastases, effect of roentgen therapy).[210, 211] These views have been confirmed by other workers on the basis of clinical studies.[212–215] In conjunction with the aforementioned data relating to "acid" and "alkaline" phosphatases,[197] it is understandable that particularly illuminating findings have been

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[199] J. P. Greenstein: J. Nat. Cancer Inst. *2*: 511 (1942).
[200] J. W. Schoonover & J. O. Ely: Biochem. J. *29*: 1809 (1935).

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[201] R. O. Bowman & H. C. Pitts: Am. J. Obst. & Gynec. *32*: 957 (1936).

[202] A. Bodansky & H. L. Jaffe: Proc. Am. Soc. Biol. Chem., 28th meet., p. 11 (1934).

[203] D. Albers: Ztschr. f. d. ges. exper. Med. *104*: 158 (1938).

[204] J. W. Schoonover: Biochem. J. *30*: 1097 (1936).

[205] H. Baumert: Ztschr. f. d. ges. exper. Med. *100*: 468 (1937).

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[206] C. C. Franseen & R. McLean: Am. J. Cancer *24*: 299 (1935).

[207] A. B. Gutman, T. L. Tyson & E. B. Gutman: Arch. Int. Med. *57*: 414 (1936).

[208] A. Astuni: Tumori n. s. *10*: 266 (1936).

[209] G. T. Harrell & S. Fisher: J. Clin. Investigation *18*: 687 (1939).

[210] H. Q. Woodard, G. H. Twombly & B. L. Coley: J. Clin. Investigation *15*: 193 (1936).

[211] H. Q. Woodard & N. L. Higginbotham: Am. J. Cancer *31*: 221 (1937).

[212] H. Lubenstein: Ztschr. f. d. ges. exper. Med. *100*: 468 (1937).

[213] T. Meranze & M. M. Rothman: Pennsylvania M. J. *41*: 1160 (1938).

[214] S. A. Wolfson, S. Reznik & L. Gunther: J. A. M. A. *116*: 1044 (1941).

[215] H. Q. Woodard & N. L. Higginbotham; J. A. M. A. *116*: 1621 (1941).

obtained regarding the serum phosphatase in patients with metastases of prostatic cancer. In these instances, high values were determined principally for the acid phosphatase in the serum, whereas this enzyme was not detected in sera of patients suffering from other bone lesions, even when the alkaline phosphatase was markedly increased.[216] This observation has been confirmed in continued work on the same subject, and the value of the estimation of acid phosphatase in serum for differential diagnosis was stressed, inasmuch as only metastases of prostatic cancer caused a rise of this enzyme activity, whereas Pagets disease or other bone pathologies are accompanied only by increased values of serum alkaline phosphatase.[217] Determinations of the serum acid phosphatase were performed in 147 patients with prostatic cancer and in 283 control cases; since in the latter normal values were always found, the rise of the acid phosphatase occurring in patients with cancer of the prostate was regarded as a very valuable aid for the recognition of bone metastases and for prognostic purposes.[218] As will be discussed in chapter VII, beneficial effects were obtained in prostatic cancer by means of orchiectomy or of estrogen administration. In this connection it is of interest that these interferences also resulted in a lowering of the serum acid phosphatase, whereas administration of androgens promoted the growth of prostatic cancer and, at the same time, caused a rise of the serum acid phosphatase.[219, 220]

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216. A. B. Gutman & E. B. Gutman: J. Clin. Investigation *17*: 473 (1938).
217. A. B. Gutman, E. B. Gutman & J. N. Robinson: Am. J. Cancer *38*: 103 (1940).
218. C. C. Herger & H. R. Sauer: Cancer Research *2*: 398 (1942).
219. Ch. Huggins & C. V. Hodges: Cancer Research *1*: 293 (1941).
220. L. F. Ajamil: Bol. Liga contra el cancer *16*: 329 (1941); abstr. J. A. M. A. *118*: 1166 (1942).

5. *Amylase.*

The presence of diastatic enzymes (amylase) in malignant tumors was demonstrated some time ago,[166] and in later work the diastatic or glycogenolytic activity of neoplasms has been more clearly defined. A high content of amylase and glycogenase has been found in Rous sarcomas,[154] and an increased glycogenolysis has also been claimed for some human neoplasms.[221] The amylase content of uterine carcinomas was not found to differ appreciably from that of normal uterine tissue, yet less differentiated tumors exhibited a higher enzymatic activity than more mature ones.[222] More detailed studies have been devoted to the amylase activity of transplanted mouse sarcomas,[223] and the analysis of their kinetics showed an optimum of 6.2 for the pH, and of 48 to 50° C for the temperature. Injection of insulin, administered to animals prior to the removal of the tumor, increased the enzyme content by thirty per cent, thyroxine caused an increase of thirteen per cent, while epinephrine was ineffective.[224] A high amylase activity has been established for the Jensen rat sarcoma, and this finding was connected with the fact that only traces of glycogen were detectable in this tumor.[225] Examinations of the amylase content in various animal tumors (rabbit carcinomas and sarcomas, rat carcinomas and sarcomas, mouse carcinomas) showed necrotic tumor parts to exhibit a greater enzymatic activity than intact areas, and slight changes of the amylase activity found in organs of tumor hosts during the terminal stages of the tumor process were regarded as being due to cachexia.[226] Recent investigations showed no appreciable difference in the amylase activity of transplanted hepatic rat tumors and normal rat liver,[159] whereas hepatic mouse tumors showed no uniform behavior. Tumors of the dilute brown strain possessed an amylase activity corresponding to that of normal liver, but the enzyme content of tumors of C3H mice was markedly increased, both as compared with normal liver or with the tumors of dilute brown mice.[160] An earlier report claimed that aqueous tumor extracts frequently inhibited diastatic enzymes,[227] but a later examination failed to reveal the presence of diastase inactivators in tumor tissues.[228]

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[221] M. Vittorio: Policlinica (sez. chir.) *37*: 6 (1930).

[222] K. Nakahori: Jap. J. Obst. & Gynec. *17*: 254 (1934).

[223] F. H. Scharles & W. T. Salter: Am. J. Cancer *20*: 613 (1934).

[224] F. H. Scharles, P. Robb & W. T. Salter: Am. J. Cancer *23*: 322 (1935).

[225] S. Edlbacher & W. Baumann: Ztschr. f. Krebsforsch. *47*: 191 (1938).

The diastatic activity of blood was found lowered in cancer patients and also in other conditions of cachexia,[229] but other workers did not observe changes of this enzymic activity unless dealing with pancreas tumors.[230, 231] In rats with Walker carcinomas a dimunition of almost fifty per cent of the serum amylase has been observed,[178] and likewise rats inoculated with Jensen sarcomas showed a decrease of the amylase activity in the blood, running parallel to progressive tumor growth. This alteration was not considered as being merely due to cachexia.[232] Another worker, however, who found a decreased serum amylase activity in fourteen out of twenty-five rabbits bearing Brown-Pearce carcinomas interpreted this decrease as being caused by nutritional disturbances.[226] An activator of diastatic enzymes has been found present in approximately seventy per cent of the urines of

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226. G. Mori: Acta dermat. *32*: 1; 25 (1938).
227. F. P. Tinozzi: Ztschr. f. Krebsforsch. *38*: 462 (1932/33).
228. E. Stolfi: Bol. soc. ital. biol. sper. *11*: 922 (1936).
229. Ch. Achard & A. Clerc: Compt. rend. Soc. de biol. *53*: 708 (1901).
230. H. H. DeNiord: Arch. Int. Med. *23*: 484 (1919).
231. L. L. Tureen: Arch. Path. *15*: 834 (1933).
232. L. Berkesy: Ztschr. f. Krebsforsch. *50*: 121 (1940).

tumor patients tested[233, 234] and this phenomenon has been considered as a helpful aid for tumor diagnosis (cf. chapter X).

Only meager experimental material has been collected as to the influence of diastatic enzymes on the tumor process; injections of a diastase preparation into tumor-bearing rats caused toxic effects frequently leading to shock and death of the animals, and tumor regressions were only rarely observed in the surviving animals. Percutaneous inunction of aqueous alkaline enzyme solutions were, however, claimed to bring about a growth inhibitition of the tumors regularly.[188]

6. *Catalase.*

The physiological significance of this enzyme, which represents an iron-porphyrin system, has been assumed to lie in the removal of hydrogen peroxide and other peroxides formed in the course of oxidation processes,[235] and hence an involvement of catalase in the disturbances of the oxidative metabolism, established for malignant tumors, might be suspected. Actually, the investigations devoted to this question have almost unanimously demonstrated a reduced catalase content in malignant tumors, and also in certain organs of tumor hosts; the establishing of a definite connection of these results with alterations of the tumor metabolism has not as yet been feasible. As far back as 1910 a diminished catalase content was found in cancerous tissue, and it is significant that low enzyme values were also observed in the apparently healthy surrounding tissues,[236] or in the metastases-free liver

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[233] P. Geréb: Ztschr. f. Krebsforsch. *36*: 115 (1932).

[234] E. Fränkel, P. Geréb & R. Simke: Biochem. Ztschr. *245*: 44 (1932).

[235] C. Oppenheimer: Biochemie, Erg.-Bd. IB, p. 842, G. Fischer, Jena 1933.

[236] F. Blumenthal & B. Brahn: Ztschr. f. Krebsforsch. *8*: 436 (1910).

of tumor hosts.[237] Several reports confirmed these findings by showing a diminished catalase activity in the liver of tumor patients,[238–240] an alteration which has been viewed by some authors as an expression of cachexia.[238, 240]

A decreased catalase activity has been established for the Rous sarcoma,[241] and particularly extensive work on catalase in relation to animal tumors has recently been carried out. These studies attempted to gain more precise information by means of a comparison of neoplasms and their parent tissues, and by investigating tumors of well defined biological properties; in this connection it was first ascertained that transplanted hepatic rat tumors exhibited a much lower catalase activity than normal rat liver and, at the same time, a considerable drop of the enzymatic activity was shown to occur in the liver of rats inoculated with hepatic tumors or with Jensen sarcomas.[159] A marked decrease of catalase was, furthermore, demonstrated in transplanted hepatic mouse tumors and in transplanted mouse lymphomas when the enzymatic activity was compared with that of the respective normal tissues, whereas the catalase values determined for two different types of spontaneous mammary tumors of mice approximately equaled those obtained in hyperplastic mammary tissue. The liver catalase activity of mice bearing various tumors was diminished in all instances, although the degree of this decrease and the time of the appearance of this enzymic alteration varied according to the type of tumor.[160] Essentially the same results were obtained when, instead of the volumetric method previously used, the catalase activity was determined by means of a titrametric method in livers of normal animals and of tumor hosts.[242] Continued investigations into the liver catalase activity of tumor-bearing rats yielded the following significant data. It was ascertained that the drop of the liver catalase is closely linked to the presence of the tumors, since their removal was followed, within a day or two, by a restoration of the normal enzymatic activity, while a second tumor inoculation again caused a catalase diminution in the liver of the same magnitude as observed following the first tumor transplantation. The possibility that a secondary inanition of tumor-bearing animals might be responsible for this enzymatic disturbance may be ruled out, since starvation from one to three days failed to produce any appreciable change in the liver catalase of normal rats. On the basis of these results, it was thought probable that the effect of the presence of the tumor on the liver catalase is caused by a toxic substance which is produced by the tumor and reaches the liver via the blood stream. No explanation can as yet be offered for the interesting observation that in the first week of tumor development a marked increase of the liver catalase takes place, whereas continuing growth of the tumors is followed by the decrease of the liver catalase level mentioned, which reaches a minimum in animals with four week old tumors, and then remains nearly stationary. Yet in this connection it seems very plausible to assume that the supposed toxic substance, responsible for the drop in the catalase content, must be accumulated in the liver until it reaches a certain threshold value before it affects the enzymic activity, and it is obvious that the time required for this process will largely depend on the rate of growth of the tumor under investigation.[243] Actually, investigations conducted on the liver catalase of mice

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[237] B. Brahn: Ztschr. f. Krebsforsch. *16*: 112 (1914).
[238] H. A. Colwell: Arch. Middlesex Hosp. *19*: 64 (1910).
[239] E. Rosenthal: Deutsche med. Wchnschr. *38*: 2270 (1912).
[240] J. Magat: Ztschr. f. d. ges. exper. Med. *42*: 95 (1924).
[241] M. C. Lewis & H. Cossman: Am. J. Physiol. *87*: 584 (1928/29).

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242. J. P. Greenstein: J. Nat. Cancer Inst. *2*: 525 (1942).

243. J. P. Greenstein, W. V. Jenrette & J. White: J. Nat. Cancer Inst. *2*: 283 (1941).

bearing various types of neoplasms have borne out the assumption outlined above inasmuch as, with very few exceptions, a pronounced parallelism was found to exist between the growth rate of different tumors and their effect of lowering the liver catalase activity. It is also noteworthy that in analogy to the effect of tumor removal, the spontaneous regression of tumors growing in certain mouse strains was followed by a return of the liver catalase to the normal.[244] Additional support for the assumption of a systemic involvement of catalase in neoplasia was furnished by the report that the activity of this enzyme was found to be decreased in the kidneys of rats and mice bearing tumors. This effect was, however, less marked than in the case of hepatic tissue.[245] Hence it may be stated that the work discussed has not only clearly established a depression of the catalase activity in most malignant tumors tested, but has also disclosed the occurrence of a systemic effect, caused by the tumor process, as expressed in a markedly reduced catalase activity of the hepatic tissue of tumor hosts. With regard to the tentative hypothesis that the latter phenomenon is evoked by a toxic substance, formed in the neoplasms and transported by the blood stream to the organs of the tumor host, it must be admitted that the nature of this supposed toxic agent is as yet unknown, and no explanation can at present be given for the fact that this agent apparently affects only certain enzyme systems, while others remain unchanged. The elucidation of these problems requires continued work, for which a solid foundation has been laid by the studies discussed.

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244. J. P. Greenstein & H. B. Andervont: J. Nat. Cancer Inst. *2*: 345 (1942).

245. J. P. Greenstein, H. B. Andervont & J. W. Thompson: J. Nat. Cancer Inst. *2*: 589 (1942).

Several workers have reported on a diminished catalase content in the blood of tumor patients,[238, 239] but later studies showed that this decrease of the blood catalase activity in the first stages of neoplasia ran parallel to the diminution of the red blood count, whereas only later stages of the disease were accompanied by a low catalase content as related to the single erythrocyte.[240, 246, 247] Likewise, in tumor-bearing animals (rabbits, rats, fowl), the decrease of the blood catalase level was found to run parallel to the progressing tumor growth and to the decrease of hemoglobin,[248] but this finding was supplemented by the statement that mice with transplanted carcinomas exhibited an increase of the blood catalase in the first stages of tumor development, and that a proportional decrease of the enzyme activity accompanied later stages of the tumor process.[249] It is noteworthy that the author of the previous communication observed irregular changes—both increase and decrease of the catalase value—in the blood of cancer patients [249] and, since preliminary experiments with tumor-bearing animals failed to reveal an appreciable change of the blood catalase activity,[243, 245] a definite connection between blood catalase and neoplasia cannot at present be regarded as proven. Finally, it may be mentioned that no characteristic change has been observed in the catalase content of urines of cancer patients,[250] a finding which is not surprising, since this enzymatic activity is chiefly linked to the presence of red and white blood cells in the urine and, accordingly, is in no way directly connected with tumor pathology in general.

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246. Zerner: Ztschr. f. Krebsforsch. *19*: 263 (1922).
247. L. Deutsch & J. Frankl: Ztschr. f. Krebsforsch. *40*: 98; 510 (1934).
248. Y. Yoshida: Verhandl. d. jap. Gesellsch. f. inn. Med. *23*: 100 (1926).
249. H. Werner: Acta Cancrol. *2*: 157 (1936).
250. M. Kahn & Ch. J. Brim: abstr. Zentralbl. f. Biochemie *18*: 32 (1916/17).

7. *Oxidases.*

These enzyme systems which, in accordance with modern concepts of their kinetics, should more appropriately be termed dehydrogenases, are most closely connected with processes of biological oxidation, and accordingly the material presented here will be supplemented by the discussion of oxidative tumor metabolism in chapter VI. The evidence so far available has only to a limited degree been able to link alterations of the tumor metabolism with definite enzyme systems, and diverging results obtained in this field may be regarded as due to the fact that individual studies have dealt with different enzyme systems and have tested them by means of different substrates or methods. A diminished oxidase content of tumor tissue has been inferred from experiments on oxidation of salicylic aldehyde, and this oxidase activity was also found lowered in apparently healthy tissue surrounding tumors.[251] Far reaching claims have been expressed as to the lack of oxidases in the nuclei of tumor cells, inasmuch as, in contrast to normal cell nuclei, neoplastic cells failed to show the ability to oxidize leucomethylene blue (Rongalite white) to methylene blue, and consequently remained unstained.[252, 253] These observations, however, could not be confirmed in a re-examination,[254] and hence neither the theoretical implications nor the possibility of deriving a method of histological tumor diagnosis from this phenomenon appear proved. Comparative determinations of the indophenol oxidase in normal human tissues, in benign and

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251. B. Brahn: Ztschr. f. Krebsforsch. *17*: 417 (1920).
252. G. E. Roskin: Ztschr. f. Krebsforsch. *35*: 140 (1932); Arch. f. exper. Zellforsch. *14*: 781 (1932).
253. G. E. Roskin & Ssolowjewa: Arch. path. Anat. u. path. Physiol. URSS *4*: 4 (1938); abstr. Am. J. Cancer *40*: 419 (1940).
254. C. A. Lang: Boll. d. lega ital. p. l. lotto contro il cancro *13*: 165 (1935); abstr. Am. J. Cancer *31*: 115 (1937).

in malignant tumors, failed to disclose any characteristic difference between these tissues[255] and studies on indophenol oxidase performed with cell suspensions of various transplanted animal tumors showed an increase of the enzyme activity running parallel to progressing tumor necrosis, a behavior which one is obviously justified in connecting to the presence of hemorrhages in necrotic tumor parts,[256] and which therefore bears no direct relation on tumor enzymology. In a previous section, alterations of the xanthine dehydrogenase (xanthine oxidase) have been discussed, but it was stressed that different types of tumors are distinguished by their enzyme activity as compared with the normal tissues from which they are derived. It may in addition be mentioned that an identicality of xanthine dehydrogenase with the enzyme aldehydrase or with the Schardinger enzyme has been made probable,[257] and hence this enzyme may be assumed to participate also in the intermediate carbohydrate metabolism. Furthermore, the xanthine oxidase activity, as measured by the reduction of methylene blue in the Thunberg test, has been found markedly depressed in the liver of mice belonging to strains susceptible to cancer, as compared with results obtained in animals of strains resistant to the disease. This alteration was believed to be partly responsible for the susceptibility to tumors, since the livers of these animals would possess an impaired ability to destroy break-down products of nucleoproteins required for tumor formation[258] (cf. chapter IX). The presence of xanthine dehydrogenase and lactic acid dehydrogenase has been demonstrated for transplanted mouse and rat tumors, and thus similar relations were found to exist as are present in normal

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255. G. Melot: Bull. Soc. chim. biol. *20*: 352 (1938).
256. G. Mori: Acta dermat. *33*: 1; 47 (1939).
257. V. H. Booth: Biochem. J. *32*: 494 (1938).
258. F. H. Figge & L. C. Strong: Cancer Research *1*: 779 (1941).

muscle.[259] Likewise, an alcohol dehydrogenase, requiring the presence of adenosinetriphosphate as coenzyme, has been shown to be present in human and animal tumors,[260] and an enzyme of great importance for carbohydrate metabolism, viz., the fumarase, which converts fumaric acid into oxymaleic acid, has been found to be very active in malignant tumors.[261] The kinetics of dehydrogenases were tested by reduction of m-dinitrobenzene and of methylene blue. The enzymes of tumors showed only a slight sensitivity against changes of the hydrogen ion concentration, in contrast to normal tissues or benign tumors, which exhibited a sharply defined optimum of the pH for the enzyme activity. This was interpreted as indicating the presence of a greater number of different dehydrogenases in the neoplastic tissues, and the optimum pH of the various enzymes was assumed to cause an overlapping.[262] No definite evaluation can as yet be given for the significance and the enzymatic character of observations, according to which alcohol-ether extracts of blood of cancer patients showed a markedly diminished capacity for decolorizing methylene blue, when compared with the behavior of blood extracts of normal persons.[263]

Only meager material is available as to the content of peroxidases in malignant tumors, that is, of enzymes acting as oxygen carriers in the presence of peroxides and of oxidisable compounds. A very low peroxidase activity was determined in animal tumors by means of the purpurogallin method, as is, incidentally, also the case in most normal organs, excepting spleen and lung.[264] When, on the other hand, a strong benzidine peroxidase reaction was reported in gastric cancers,[265] this might have been due to a secondary factor detected in various animal tumors in which the benzidine peroxidase activity showed great variations. In general, it was found to increase in consequence of hemorrhages occurring in the tumors,[266] thus apparently reflecting the presence of extravasated blood. A close relationship with peroxidases can be assumed of some enzymes connected with pigment production, and in this connection it is not surprising that tyrosinase has been shown to be present in melanotic tumors,[267] and also in the urines of patients suffering from melanoblastomas.[268]

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[259] E. Boyland & M. E. Boyland: Biochem. J. *29*: 1097 (1935).
[260] J. Lehmann: Biochem. Ztschr. *272*: 144 (1934).
[261] J. H. Quastel: Nature, London *132*: 101 (1933).
[262] B. Borghi: Tumori n. s. *11*: 231 (1937).
[263] E. Rousseau: Compt. rend. Acad. d. sc. *205*: 1101 (1937).
[264] G. Bancroft & K. A. C. Elliott: Biochem. J. *28*: 1911 (1934).

8. *General Views on Tumor Enzymology.*

While the results surveyed in the foregoing have by no means furnished definite evidence for a uniform behavior of malignant tumors with regard to their enzymatic activity, and only few data have indicated the occurrence of systemic changes of enzymatic processes in neoplasia, some authors have expressed opinions which assign a central position to enzymes in tumor pathology. Such theories have been mentioned before, inasmuch as a prevalence of hydrolytic enzymes over oxidative enzymes has been supposed to characterize malignant tumors[192] and a similar viewpoint regarded a deficiency of oxidizing enzymes as being responsible for an accumulation of unsaturated compounds, which in turn would disturb the normal equilibrium required for orderly cell proliferation, and thus cause neoplastic growth.[269] Furthermore, reference is made to theories which deal with one special type of neoplasia, i.e., the filterable fowl tumors. As will be mentioned in chapter IX, several

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265. S. Ito: Tr. Jap. Path. Soc. *21*: 754 (1931).
266. G. Mori: Acta dermat. *33*: 25; 51 (1939).
267. C. Gessard: Compt. rend. Acad. d. sc. *136*: 1086 (1903).
268. O. Gross: Deutsche med. Wchnschr. *45*: 488 (1919).
269. M. Copisarow: Protoplasma *22*: 137 (1934).

authors ascribed an enzyme nature to the agent present in these tumor filtrates with the property of producing tumors.[270-272] It has even been believed possible to apply such assumptions to neoplasia in general, and to base the cancer etiology on the existence of a "tumor enzyme." This enzyme, it was supposed, might cause irreversible changes of the metabolism in a normal cell and thus transform it into a malignant cell; the agent was supposed to be ubiquitous in an inactive form, and stimuli known to be carcinogenic were held to be capable of converting the enzyme into an active form, or, as far as animal experiments with transplanted tumors are concerned, the enzyme together with the tumor inoculum would be transmitted into the new host.[273] Yet, as will be discussed in chapter IX, the existence of a noncellular agent—be it an enzyme or a virus—which can be isolated from neoplasms and which is capable of causing tumor formations has up to the present never been proved, with the exception of the instances of "infectious" fowl tumors and some similar mammalian neoplasms. A purely speculative character may be ascribed to a theory which holds an undefined enzyme, which is activated by the influence of the central nervous system, to be responsible for tumor genesis.[274]

Summary.

This survey of tumor enzymology has doubtlessly revealed the occurrence of enzymatic alterations in malignant tumors and in organs of tumor hosts, but the data available represent a rather vague outline which continued work will have

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[270] Teutschlaender: Ztschr. f. Krebsforsch. *24*: 223 (1927).
[271] E. Fränkel: Ztschr. f. Krebsforsch. *25*: 407 (1927).
[272] F. Pentimalli: 1st Internat. Cancer Congress, Madrid *2*: 81 (1933).
[273] F. Blumenthal: Ergebnisse der experimentellen Krebsforschung und Krebstherapie, Leiden 1934.
[274] M. Mühlmann: Ztschr. f. Krebsforsch. *23*: 385 (1926); Acta Cancrol. *2*: 165 (1936).

to fill in regarding numerous important details. In this connection it has recently been pointed out—with justification—that much of the older work on enzyme problems in cancer pathology is hardly available for a precise interpretation on account of the lack of comparative data relating to the enzymatic activity in the parental tissue from which the tumor tested has been derived. The establishing of such comparisons is the more imperative, since a great many studies clearly demonstrated that the same enzyme may be affected in varying manner in different types of neoplasia, whereas, on the other hand, far reaching alterations of one enzyme may be associated with normal activities of other enzymes in the same tumor. Although definite conclusions would be premature on account of the steady progress of our knowledge, the general impression may be gained from the results established up to the present that the tumor process, only to a lesser degree, involves changes of those enzymes which attack the more complex body constituents (proteinases, lipases, amylase), whereas enzymes participating in the intermediate metabolism are much more markedly affected.

It has in some instances been possible to detect a systemic influence of the neoplastic process on enzymatic activities of organs, far from the site of the tumor, but analyses of these effects showed them for the most part to be consequences of the tumor growth, since a restoration to normal relations took place following tumor removal. While detractions from the significance of these observations, which may furnish a satisfactory explanation of some clinical symptoms apparent in neoplasia (cachexia), should not be made, it must be stressed that another important approach to the problem of tumor enzymology is still awaiting a systematic elaboration. This refers to the question whether, and in what manner, early stages of tumor development are associated with disturbances of enzymatic processes. Experiments of this kind are rendered possible today by virtue of the chemical or physical carcinogens, which permit a continuous investigation of the treated animals from the start of the experiments until the tumor has been induced. Investigations of this kind have only infrequently been conducted and the results must be considered as being only of a preliminary nature, but more extensive elaboration may be expected to yield important clues as to the mechanisms of carcinogenesis.

It is obvious that many enzymological studies have been undertaken with practical aims in mind, that is, by striving to utilize for diagnostic purposes enzymatic deviations observed in the course of neoplastic diseases. Naturally, this trend of work extended particularly to enzymes present in the blood or serum of tumor hosts. In this regard no enzymatic alterations which could serve as a basis of diagnostic tests for the presence of malignant tumors in general could be detected; nevertheless significant findings have been obtained relating to special types of neoplasia, such as are exemplified by quantitative and qualitative changes of phosphatases in patients with bone metastases of prostatic cancer, or with other bone tumors. The data established for this enzyme proved helpful for differential diagnosis and prognosis, and it is within the range of possibility that studies of other enzymatic activities may be correlated with different forms of neoplasia in a similar manner. Little need be said of the therapeutical uses of enzymes in tumor pathology for, even granted that enzymatic alterations may eventually be disclosed as being intimately connected with tumor development and growth, it would be an extremely primitive concept if administration of one enzyme or the other were to be proposed as an effective means of counteracting an enzymatic disturbance of necessarily intra-cellular nature. As a matter of fact, no instance is known in general pathology, with the exception of gross intestinal deficiencies of enzymes, in which an enzyme therapy has proven useful.

CHAPTER V

NUTRITION AND VITAMINS

OPINION as to the significance of nutrition or of various diets as factors which determine the course of diseases has in general undergone considerable fluctuations during the last century. During the pre-anatomical period of humoral pathology considerable influence of a rather mysterious nature was ascribed to nutritional factors. Many biological processes and most pathological changes were regarded as being dependent on the maintenance or on a disturbance of the composition of the body fluids into which the food materials were supposed steadily to enter. However, with the development of cellular pathology, on the one hand, and of physiological chemistry, on the other, a complete revision of these views took place. This was brought about by the recognition of the fact that the most varied and complex food stuffs can be reduced by analytical methods to a few categories of vital alimentary substances and, in addition, that these substances undergo an enzymatic cleavage in the intestinal tract, resulting in the formation of comparatively simple compounds. These products were shown to have lost, on the one hand, any special relationship to the original food material ingested and, on the other hand, to be re-synthesized into new products typical for the organism of which they are destined to be constituents. The latter fact was especially liable to shake the belief in the significance of qualitative variations of dietary regimes, as long as their quantitative composition remains the same. The climax, but at the same time the turning point, of this trend of thought was represented by the concept of the isodynamic effect of food constituents which are equal in their caloric value. This law was soon found to be contradicted by recognition of the fact that the ingestion of a certain minimum amount of protein is required for the maintenance of health and normal growth, and thus it was demonstrated that at least one food constituent cannot be replaced by others of identical caloric value. Continued investigations of the problem of nutrition led to experiments in feeding animals with simple, almost chemically pure substances, but these attempts encountered surprising difficulties and—together with clinical observations—eventually paved the way for the discovery of accessory food substances, viz., the vitamins. Moreover, it has been clearly established that unbalanced diets, even if sufficient from a caloric viewpoint, may exert notable influences on the metabolism, and from these observations valuable information has been derived, not only for the understanding and treatment of metabolic pathologies sensu strictiori (diabetes, gout, etc.) but also of diseases with a less definite relationship to metabolic processes, as, for instance, tuberculosis.

The circumstances, outlined in the foregoing, may serve to explain the great interest which, for a long time, has been devoted to nutrition in connection with the cancer problem. Yet, it shall not be denied that a great number of studies performed in this field, and particularly the claims advanced, were caused by a less rational factor, that is, by the failure to establish an intrinsic cause for cancer by means of histological, bacteriological or chemical methods, and hence the attention was shifted to external causative factors, which might be represented by qualitative or quantitative changes of the nutrition. A clear distinction must be made between those studies and theories which have been concerned with the effect of dietary factors on the development of neoplasia, and those concerned with nutritional measures affecting the growth of existing tumors. The latter trend, as is generally the case in oncology, does not necessarily bear on the problem of tumor etiology, but instead has been utilized for practical purposes, inasmuch as it has served as a foundation for the establishing of dietary regimes believed to be useful in human neoplasia. The very numerous data collected in these fields have formed, in recent years, the subject of an extensive monograph by Hoffman,[1] who reviewed the literature and also presented his own statistical findings regarding cancer and diet. Later Caspari,[2] who contributed essentially to the elaboration of this branch of cancer research, reported the results of his survey of these problems in a more condensed form.

The following presentation will be divided into three parts, the first of which will deal with the major foodstuffs, insofar as their qualitative or quantitative variations affect development and growth of malignant tumors. The second part will be devoted to similar aspects concerning the vitamins; and the third will briefly survey dietary regimes which have been proposed for the treatment of neoplasia.

1. *Major Food Constituents.*

Food quantity and food requirement. In regard to the relation between food quantity and tumor growth, the prevailing majority of authors have expressed the opinion that food restriction is followed by a depression of neoplastic growth. This fact, incidentally, was already known to physicians in antiquity [cf. 3] who advocated a starvation treatment ("fames cura") for cancer. This belief has been maintained in popular medicine and emerged in medical opinions of the eighteenth century (bibliography, cf.[4]). In modern times, the growth-inhibiting effect of undernourishment has been frequently found for transplanted animal tumors,[4, 5–9] regardless of whether the food deficiency has been of a caloric nature, or related to a lack of vitamins or minerals,[4] and only a few dissenting opinions have been recorded.[10] Conversely, overfeeding of the animals was followed by a stimulation of transplanted tumors.[4, 11] An older study considered the question of spontaneous food intake by tumor-bearing animals, and it was observed that the food requirement of these animals in the first stages of tumor development approximately equalled that of normal animals, whereas the appetite of the tumor-bearing animals gradually decreased during the later stages of the disease; at the same time, gains in body weight stopped, though the tumors increased in size.[12] More recently, a thorough analysis of the effect of underfeeding on transplanted mouse tumors has been performed with the following results. Caloric restriction to fifty per cent of the normal caused a considerable growth inhibition, and

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[1] F. L. Hoffman: Cancer and Diet, William & Wilkins, Baltimore 1937.

[2] W. Caspari: Nutrition et Cancer, Herman & Co., Paris 1938.

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[3] Cornelius Nepos: Vitæ; Atticus, Cap. XXI, XXII.

[4] E. Friedberger & I. Grünstein: Ztschr. f. d. ges. exper. Med. *62*: 344 (1928).

[5] E. Moreschi: Ztschr. f. Immunitätsforsch. u. exper. Ther. *2*: 651 (1909).

[6] R. Guastalla: Arch. per. l. sc. med. *53*: 761 (1929); abstr. Cancer Rev. *6*: 19 (1930).

[7] G. Vecci & A. Vecci: Arch. per l. sc. med. *53*: 752 (1929).

[8] K. Uletzko-Stroganowa: Ž. Akuš. i. Ginek. *44*: 265 (1933); abstr. Ztschr. f. Krebsforsch. *40*: Ref. 175 (1934).

[9] F. Ottensooser: Bol. lega ital. p.-l. lotta contro il cancro *7*: 197 (1933); abstr. Am. J. Cancer *22*: 910 (1934).

[10] S. Fränkel, B. Bienenfeld & E. Fürer: Wien. klin. Wchnschr. *30*: 1131 (1917).

[11] E. Friedberger & F. Heim: Ztschr. f. Krebsforsch. *27*: 490 (1928).

[12] F. Medigreceanu: Berlin. klin. Wchnschr. *47*: 772 (1910).

tumors of the underted animals showed a diminished virulence when re-inoculated; caloric restriction to sixty-six per cent was followed by lesser but still recognizable effects, whereas a restriction to eighty per cent proved ineffective.[13] In continuing this work it was stressed that a caloric restriction by itself caused a reduced growth of mouse sarcomas 180, without it being necessary that a deficiency of certain food constituents should exist, although the ingestion of protein was considered to be particularly important for tumor growth. In this connection emphasis has been placed on the circumstance that various therapeutic measures, which it is claimed are of a specific nature, in reality may depend on the indirect effect of decreasing the food intake of the animals treated. For instance, the restraining effect on tumors of hypophysectomy (cf. chapter VII) was believed to be caused not so much by endocrine mechanisms, but rather by an ensuing caloric restriction and a consequent protein starvation of the hypophysectomized animals.[14] According to one investigator, no effect on neoplastic growth resulted from the fasting of mice and rats bearing transplanted tumors, nor was the transplantability of tumors derived from fasting animals impaired.[15] This indicates that short-period starvation is much less effective than chronic undernourishment, apparently because the growth character of neoplasms permits them to retain food reserves needed by the body for a longer time.

Very illuminating results were obtained in work dealing with the influence of food restriction on spontaneous animal tumors. In one of the oldest investigations, the effect of nutrition on the recurrence of partially excised spontaneous mouse tumors was tested. Following such a procedure, recurrences appeared in eighty-three per cent of the animals on a normal diet, but only in forty-one per cent of underfed mice; likewise re-inoculation of extirpated tumors was successful in forty-one per cent of undernourished animals, as compared with sixty-eight per cent in control mice.[16] One author reported that the appearance of spontaneous mouse tumors apparently was not affected by quantitative changes in the dietary regimes of the animals,[17] but later work definitely established that significant effects result from the restriction of the food intake of animals. In underfed mice the appearance of both spontaneous mammary cancer and of tumors induced by benzpyrene was delayed, and the total number of tumors was decreased as compared with animals on a normal diet. It was, however, stressed that different factors govern tumor induction and tumor growth, since the growth of already developed tumors was the same in underfed and in normally fed animals; only when normally fed animals with tumors were subsequently underfed, the tumor growth rate decreased.[18] Essentially the same results were obtained in continued work devoted to the influence of calorie-restricted diets on the appearance of spontaneous mammary cancer and on the tumor induction by means of injection of benzpyrene in mice. It was emphasized that the diet employed contained the same amounts of proteins, fats and vitamins as the regime used for the control animals; the experimental animals were in excellent health, though smaller than the controls, and their life span was even longer than that of tumor-free mice kept on an unrestricted diet.[19] Similar effects were observed in virgin mice of the C3H strain by using a diet in which the

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[13] F. Bischoff, M. L. Long & L. C. Maxwell: Am. J. Cancer *24*: 549 (1935).

[14] F. Bischoff & M. L. Long: Am. J. Cancer *32*: 418 (1938).

[15] I. P. Michtchenko: Acta med. URSS *2*: 104 (1939); abstr. Ztschr. f. Krebsforsch. *50*: Ref. 435 (1940).

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16. P. Rous: J. Exper. Med. *20*: 433 (1914); Bull. Johns Hopkins Hosp. *26*: 146 (1915).
17. L. Hill: Lancet *1*: 966 (1931).
18. A. Tannenbaum: Am. J. Cancer *38*: 335 (1940).
19. A. Tannenbaum: Cancer Research *2*: 460 (1942).

intake of carbohydrates and fats was reduced to one-third of the total calories ingested; adequate amounts of proteins, vitamins and minerals were provided by the regime. Whereas animals fed an unrestricted diet showed a spontaneous incidence of mammary tumors in sixty-seven per cent of their number, no tumors at all developed in mice receiving the restricted diet.[20] Likewise, rats placed, after weaning, on a diet with restricted calories, but of high quality, did not develop any malignant tumors during the period of observation (600 days), in contrast to control animals in which neoplasms (mostly lymphosarcomas of the lungs) were frequently encountered. In these experiments, too, the restricted diet retarded the somatic growth and brought about a considerably greater longevity of the animals.[21, 21a] It is evident that great importance must be attached to these animal experiments, with the expectation that their continued elaboration may lead to valuable data also applicable to the nutrition of human beings, both with regard to longevity and to cancer prevention.

Processing and composition of food. By plotting curves of normal growth of animals, a significant effect of food processing has been demonstrated on the growth rate, when diets with equal values were employed. Raw food was found to result in the highest growth rate and overcooked food in the lowest. It was stressed that these effects were independent of the water and vitamin content of the food, but were probably to be explained by a denaturation of proteins, due to the prolonged cooking.[22] Analogous effects on the growth of transplanted tumors were observed from the feeding of differently processed diets; overcooked food depressed the yield of tumor inoculations from 71.8 per cent to 42.9 per cent or from 100 per cent to 66.6 per cent, respectively, and conversely, the duration of the life of the tumor-bearing animals receiving the overcooked diet was prolonged to an average of thirty-one days, as compared with twenty-six days in control animals. Similarly, variations in the composition of diets which affected the normal growth rate in a different manner influenced the tumor growth in the same order. Thus, feeding of bread, which resulted in a slow normal growth, caused a much lower rate of neoplastic growth than feeding of eggs, viz., a diet of higher nutritional value.[4] Nevertheless, there is some doubt as to whether the high nutritional value and the accompanying stimulation of tumor growth which was observed of raw food is not, after all, caused by the higher vitamin content of the diet, and this objection is sustained by the fact that no experiments were reported in which overcooked food was supplemented by vitamins. At any rate, the practical conclusion drawn from these experiments, to avoid an excess of raw food in neoplasia,[23] is deserving of attention. A diet of deficient composition has likewise been shown to inhibit the appearance of tar lesions in mice. Animals fed solely on oats and water developed papillomas only in thirty per cent of the cases, as compared with seventy per cent in animals fed oats and milk.[24] A diet consisting of cow milk, which may be used for the experimental production of anemia, has been found to depress the growth rate of Flexner-Jobling rat carcinomas; since, however, supplementary feeding of iron and copper prevented the development of the anemia, but did not cancel the tumor-inhibiting effect of the diet, the latter phenomenon was most likely an expression of general undernourishment.[25]

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[20] M. B. Visscher, Z. B. Ball, R. H. Barnes & I. Sivertsen: Surgery *11*: 48 (1942).

[21] J. A. Saxton, Jr.: N. Y. State J. Med. *41*: 1095 (1941).

[21a] C. M. McCay: Tr. & Stud. College Physic. Philadelphia *10*: 1 (1942).

[22] E. Friedberger: München. med. Wchnschr. *73*: 1017; 1069 (1926); Klin. Wchnschr. *5*: 1966 (1926).

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[23] W. Caspari: Ztschr. f. Volksernährung *6*: 38 (1931).

[24] D. Rochlin: Vestn. rentgenol. *4*: 211 (1926); abstr. Cancer Rev. *3*: 117 (1928); Virchows Arch. f. Path. *264*: 458 (1928).

Proteins. One of the most frequently debated questions as to alimentary influences on neoplasia is the alleged relation between cancer development and high protein content of the diet, or, more precisely, the claim that a diet rich in meat favors tumor development to a much higher degree than a vegetarian diet which is comparatively poor in proteins. This opinion has been principally advocated by lay propaganda which usually has the tendency to over-simplify biological problems and, in this manner, charged the increasing frequency of neoplasia to an excessive intake of meat products. To support this view the following circumstances have been enumerated: on the one hand it was claimed that the populations of countries with a prevailing vegetarian nutrition (China, India) showed a lower cancer incidence compared with other populations. On the other hand, it was alleged that a higher tumor frequency prevails among socially privileged population strata, and it was suggested that this is connected with the use of meat in larger quantities. Finally, the nutrition of populations lacking meat during wars and famines was believed to be accompanied by a reduction of neoplastic diseases. Closer scrutiny reveals these arguments to be for the most part unfounded; variations in the geographical distribution of cancer are based in part on statistics of doubtful reliability, partly to be explained by factors other than differences in the meat content of the diet. The more frequent tumor incidence among affluent population groups has been disproved[26, 27] and, in general, careful statistical analyses reveal a cancer frequency independent of quantitative variations of the meat consumed.[1, 28] Moreover, direct evidence of animal experiments speaks against a tumor-promoting effect of meat ingestion. Mice were divided into two groups, one of which was fed a purely vegetarian diet for three months, while the other received only raw meat; subsequent tumor inoculations resulted in a tumor yield which was ten per cent higher in the first than in the second group.[29] Other investigators failed to observe an effect on the growth of transplanted tumors from a meat-free diet,[30] and negative results have been obtained as to the influence of meat diets on the development of cancer.[31] Only one statement to the contrary has been made, implying a growth stimulation of transplanted rat sarcomas in meat-fed animals.[32] On the other hand, certain forms of neoplasia appear to be impeded by a protein-rich diet; as will be detailed later in connection with vitamin B, the formation of certain liver tumors in rats could be prevented frequently, if the diet was rich in casein and certain Vitamin B factors.[33] These findings have been confirmed,[34] yet it must be borne in mind that this protection of a protein-rich diet against tumor development has so far been established only for one instance of carcinogenesis, namely the production of liver tumors by means of butter yellow, and it is wholly conceivable that this phenomenon depends on special processes of detoxication which are of little or no significance in the formation of other types of tumors. In summarizing, it may be stated that the alleged

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[25] K. Sugiura & S. R. Benedict: Am. J. Cancer *26*: 115 (1936).

[26] H. Weinberg: Ztschr. f. Krebsforsch. *11*: 302 (1911).

[27] A. Grotjahn & I. Kaup: Soziale Pathologie, p. 375 (1923).

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28. S. Peller: Ztschr. f. Krebsforsch. *36*: 217 (1933).
29. J. Danysz & Z. Skoczynski: Compt. rend. Soc. de biol. *74*: 1114 (1913).
30. K. Sugiura & S. R. Benedict: J. Cancer Res. *10*: 309 (1929).
31. M. A. François: Cancer, Bruxelles *7*: 241 (1931).
32. M. Iwano: J. Biochem. *4*: 481 (1924/25).
33. C. J. Kensler, K. Sugiura, N. F. Young, C. R. Halter & C. P. Rhoads: Science *93*: 308 (1941).
34. J. A. Miller, D. L. Miner, H. P. Rusch & C. A. Baumann: Cancer Research *1*: 699 (1941).

danger of cancer formation in consequence of an increased meat or protein ingestion has not found any justification from experimental work or clinical observations. Accordingly, the proposal put forward by some authors a long time ago [35-37] to keep the diet of cancer patients as free of meat as possible does not seem warranted, and successes reported from this measure most likely are to be interpreted as consequences of a general undernourishment. Actually, clinical observations indicated no inhibition of tumor growth in patients when the protein ingestion was restricted,[38] and it is noteworthy that analytical determinations showed no influence of a protein-poor diet on the protein content of animal tumors.[39]

An entirely different problem is represented by the effect on the neoplastic process which ensues from diets containing only incomplete proteins, that is, proteins, the molecule of which is deficient in certain amino acids which are of vital importance for normal growth and health and cannot be synthesized in the animal or human body. In accordance with numerous observations which show severe curtailments of somatic growth to be accompanied by depression of neoplastic growth, diets which contain glutein [40] or gelatin [41] as the only source of nitrogen have been shown to impede the tumor process. Tumor inoculation into animals fed such diets resulted in a reduced number of takes and in a diminution of the growth rate of developing tumors. This effect was particularly marked when animals were fed plant pro-

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35. Corput: Bull. d. soc. med. d. Garda 1883; quot. loc. cit. 4.
36. H. Jacubasch: Berlin. klin. Wchnschr. *23*: 861 (1886).
37. F. W. Hopman: Ztschr. f. Krebsforsch. *14*: 84 (1914).
38. A. Lumière: 1st Internat. Cancer Congress, Madrid *2*: 1008 (1933).
39. B. Kellner & B. Lustig: Biochem. Ztschr. *254*: 214 (1932).
40. J. E. Sweet, E. P. Corson-White & G. J. Saxon: J. Biol. Chem. *15*: 181 (1913).
41. L. B. Mendel & T. B. Osborne: Ztschr. f. physiol. Chem. *29*: 307; 318 (1912).

teins, which are deficient in hexone bases, substances vitally required for growth (cf. chapter II) and, accordingly, tumor inoculation was successful in only 11 per cent of animals kept on this diet, as compared with 75 per cent in control animals.[40] It may be mentioned that, conversely, a promotion of tumor growth has been disclosed to follow feeding of one of the hexone bases, namely, arginine[42] (cf. chapter II). On the other hand, the retardation of tumor growth observed in animals receiving gelatin as the sole source of protein may be connected with the lack of cyclic amino acids in this protein, and a growth depression of transplanted tumors and a prolongation of life of the tumor hosts resulted from a gelatin diet.[43] Other workers found diets containing tryptophane-deficient proteins to exert a more detrimental influence on the general health of tumor-bearing mice and rats than on the tumors themselves, and consequently the animals succumbed at an early time to this alimentary deficiency.[44] Apparently, these observations indicate that the tumors are capable of utilizing reserves of cyclic amino acids in the body at the expense of normal tissues.[2] In one study, a moderate retardation of the growth of Jensen rat sarcoma was observed when the animals received a diet free of the diamino acid lysine,[45] but other workers established a much greater effectiveness of dietary regimes deficient in lysine. Normal growth of mice, as well as growth of transplanted and spontaneous mouse tumors, were greatly impeded in animals fed heated milk powder or gliadin, viz., proteins of a very low lysine content. This growth restriction was reversible, since addition of lysine or of the lysine-rich protein glutenin was followed by an increase of the growth rate; tryptophane

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42. E. Gilroy: Biochem. J. *24*: 1384; 1659 (1930).
43. E. Centanni: Tumori *2*: 265; 466 (1912).
44. J. C. Drummond: Biochem. J. *11*: 325 (1917).
45. R. Courrier & G. Coste: Compt. rend. Soc. de biol. *115*: 631 (1934).

deprivation of the animals failed to influence tumor growth in these experiments.[46] Significant results have been announced regarding the presence of cysteine in the diet, inasmuch as a deficiency of this amino acid markedly depressed the tumor yield, following inoculation of Flexner-Jobling carcinoma into rats.[47] Furthermore, it was observed that a cysteine-free nutrition impeded the growth of spontaneous mammary cancers in mice; a similar effect of cysteine deprivation was also apparent as to the growth of normal young rats, and both the normal and the neoplastic growth rates could be restored, when cysteine or glutathione were added to the deficient diet.[48] More recently, four-weeks old virgin mice of the C3H strain were divided into two groups, one of them kept on a high-cysteine diet, the other on a low-cysteine diet. Whereas all animals of the first group showed mammary tumors after ten months, not a single tumor was found even after fifteen months in the mice receiving the low-cysteine diet.[48a] These findings are in satisfactory agreement with the relations outlined in other places of this book (cf. chapters II, IV, VI and IX), which furnish clear evidence that sulfhydryl compounds are indispensable requirements for processes of growth, enzyme activity and metabolism. In this connection it is especially noteworthy that the growth inhibition of normal animals which were treated with various organic carcinogens (cf. chapter II) could be cancelled, when the animal received sufficient quantities of sulfhydryl compounds in the diet. It was reasonable to assume that a detoxication of the carcinogens, which takes place in the body, requires and uses up sulfhydryl compounds, in this manner depriving the organism of a vital factor of growth. Furthermore, it has been shown in experimental studies that cysteine and other sulfhydryl compounds are likewise required for the maintenance of neoplastic growth. In addition to the above mentioned experiments with transplanted and spontaneous animal tumors it has been convincingly proven that cysteine or sulfhydryl deficiency impairs the tumorigenic action of butter yellow. In rats receiving butter yellow together with a diet low in cysteine content, the appearance of hepatomas was considerably delayed and the percentage of tumors was reduced, as compared with the response of animals kept on a high-cysteine diet during the administration of the carcinogen.[49] The processes involved may probably be interpreted in the following manner. After administration of these carcinogens a certain amount of sulfhydryl compounds are used by the body for purposes of detoxication; when the body reserves of these substances are exhausted during the preparatory phase of carcinogenesis, and when no additional supply of sulfhydryl compounds is provided by the diet, the resultant deficiency prevents initiation of neoplastic growth, because of the lack of these indispensable substances.

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[46] C. Voegtlin & M. E. Maver: Pub. Health Rep. *51*: 1429; 1436 (1937).

[47] A. Goerner & M. Goerner: Am. J. Cancer *16*: 360 (1932).

[48] C. Voegtlin, J. M. Johnson & J. W. Thompson: Pub. Health Rep. *51*: 1689 (1937).

[48a] J. White & H. B. Andervont; quot. C. Voegtlin: J. Nat. Cancer Inst. *2*: 309 (1942).

In studies described in chapter II, the effect of a great many amino acids on tumor growth has been tested and from animal experiments it was concluded that cysteine, glutathione, proline and alanine bring about a reduction of tumor growth.[50] On the basis of these findings a therapeutical use of these amino acids was proposed, and favorable effects were claimed to result from peroral, or parenteral, administration of the compounds named. Yet the lack of corroborating reports and the existence of conflicting evidence render a definite evaluation of these statements impossible, and this applies still more to a recommendation of obscure theoretical foundation, namely, of peroral administration of globulins, prepared from tumor tissue, for treatment of cancer.[51]

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[49] J. White & J. E. Edwards: J. Nat. Cancer Inst. *2*: 535 (1942).

[50] F. Vlès, A. de Coulon & J. L. Nicod: Compt. rend. Acad. d. sc. *197*: 350 (1933).

Carbohydrates. The preeminent part played by carbohydrates in tumor metabolism is reflected in the significance of these substances as nutritional factors in the development and growth of malignant tumors. Most experiments with animals demonstrated a promotion of the growth of transplanted tumors in animals kept on a diet rich in carbohydrates.[9, 52–54] Frequently the enhanced tumor growth resulted in a shortened life of these tumor hosts and, as described in chapter II, similar phenomena were observed in consequence of parenteral carbohydrate administration. On the other hand, animals which received a nutrition poor in carbohydrates exhibited an impeded development of subsequently inoculated tumors,[52] and chemically induced tumors appeared to be affected in a similar manner since carbohydrate-rich diets were reported to enhance the cancerization of mice treated with tar.[55] A special form of a nutrition of predominant carbohydrate nature is the use of a diet consisting exclusively of oats, and rats receiving this regime were found to develop lesions on the base of the tongue, which eventually became epitheliomas.[56] While it must be admitted that a contributing factor to the appearance of these neoplasms must be seen in the mechanical traumas exerted by the oat grains, the carbohydrate flooding of the body, resulting from the oat diet, may be regarded as a predisposing factor for

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51. H. Rubens-Duval: Bull. Assoc. franç. p. l'étude du cancer *19*: 654 (1930); Progrès méd. *1*: 609 (1931).
52. E. N. van Alstyne & S. P. Beebe: J. Med. Res. *24*: 217 (1913/14).
53. M. Händel: Ztschr. f. Krebsforsch, *21*: 288 (1924).
54. P. Rondoni: Klin. Wchnschr. *5*: 465 (1926).
55. M. Kawamura & Y. Kamikawa: Tr. Jap. Path. Soc. *20*: 668 (1930).
56. H. Stahr: Beitr. z. path. Anat. u. allg. Path. *61*: 169 (1915).

carcinogenesis, since development of tar cancer was accelerated also in mice fed on an oat diet.[57] Additional evidence of the importance of carbohydrates for malignant tumors may be derived from analytical determinations which indicated an increase of the sugar content of neoplasms in animals receiving carbohydrate-rich food.[39, 58] Reference must be made, however, to some contradictory statements: no influence of a carbohydate-poor diet was seen on the growth of transplanted rat tumors,[59] and another investigator found glucose-rich diets to reduce the number of tumors in mice painted with tar; moreover, this regime lessened the frequency of metastases and prolonged the life of the animals.[60] As a possible explanation of these discrepancies, it may be pointed out that the first report merely seems to confirm the fact that existing tumors frequently are not influenced by measures which are effective prior to tumor development. The results of the second investigation may be understood in the light of the following circumstances. Alimentary factors influence the tumor process in a two-fold way; on the one hand, they provide nutritional material required for the tumor growth and thus may enhance the neoplastic process; on the other hand, they may increase the resistance of the body against carcinogenic stimuli, and hence counteract development of neoplasia. Why one or the other reaction to carbohydrates prevails in different experiments cannot be decided as yet, but this may depend on variations of the experimental arrangements, or the qualities of the animals employed, questions which still await a definite clarification.

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57. M. Eiger & E. Czarnetzki: Ztschr. f. Krebsforsch. *33*: 296 (1931).

58. C. F. Cori & G. T. Cori: J. Biol. Chem. *64*: 11; 397 (1925); J. Cancer Res. *12*: 301 (1928).

59. J. C. Krantz, Jr., R. Musser, C. J. Carr & W. G. Harne: Am. J. Cancer *30*: 332 (1937).

60. K. A. Vannfält: Upsala läkaref. forh. *42*: 245 (1936); abstr. Am. J. Cancer *36*: 466 (1939).

It is probable that for the reasons mentioned therapeutical measures based on a possible curative effect of carbohydrate restrictions in the diet of cancer patients [61] have met with little success, and the same applies to proposals to use carbohydrate-poor diets in combination with hormones, affecting the carbohydrate metabolism,[62-64] which latter treatments will be detailed in chapter VII.

Lipids. In view of the significance of lipids in tumo pathology, as outlined in chapter II, it is understandable that great interest has been placed on the nutritional value of these substances in connection with neoplastic diseases. However, the results of these investigations are rather disappointing due to many discrepancies, one of the causes of which may be the varying vitamin content in most natural fats; consequently, some effects observed of diets rich in fats may in reality have been due to the presence of fat-soluble vitamins. From a chronological viewpoint, Freund and Kaminer [65] were the first to ascribe to food fat a fundamental significance for cancer development and growth. As will be detailed in chapter X, the theory of these authors presumed the formation of a "cancer-cell-protective factor" from animal fat, particularly tripalmitin, in the intestinal tract of cancer patients, or of persons predisposed to neoplasia. In support of this theory, reference was made to animal experiments which showed the tumor yield of inoculations of mouse carcinoma to be depressed from one hundred per cent to fifty per cent, when the animals received a diet free of pal-

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61. W. Nakahara, N. Hashimoto & R. Ikeda: abstr. Am. J. Cancer *22*: 414 (1934).
62. H. D. v. Witzleben: Klin. Wchnschr. *4*: 2115 (1925).
63. F. Silberstein, J. Freud & T. Révész: Wien. klin. Wchnschr. *38*: 356 (1925); Schweiz. med. Wchnschr. *62*: 153 (1932).
64. W. Susman: Brit. M. J. *2*: 794 (1931).
65. E. Freund & G. Kaminer: Biochemische Grundlagen der Karzinomdisposition, Springer, Vienna 1925.

mitin.[66] Such a diet was also found to inhibit the development of tar cancer in mice,[67] though it must be remarked that the latter experiments comprised only a small number of animals. The results of some other studies indicated an enhancement of tar cancer development in mice [68, 69] or in rabbits [70] when a diet rich in butter, or other fat, was administered to the animals. Yet other investigators observed that a regime containing forty per cent of butter markedly depressed the development and growth of Flexner-Jobling rat carcinoma, whereas rat sarcomas were not influenced by this diet, nor was any effect noticed from the feeding of a diet free of fats.[71] In direct contrast to Freund's theory, it was shown that a liberal palmitin ingestion markedly impeded the growth of transplanted tumors,[72] and some workers completely denied that diets, either rich or free of fat, affected the growth of rat tumors.[73] However, some experimental support for Freund's proposal to replace animal fat in the diet of cancer patients by vegetable fat may be derived from the findings that peroral administration of sesame oil strongly impeded the growth of transplanted tumors.[74] On the other hand, the growth rate of rat carcinosarcoma 256 was found to be lower in animals receiving a cod liver oil diet than in those fed cocoanut oil, and it was discovered that the presence of fatty acids with longer chains in the cod liver oil was re-

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66. E. Freund, B. Lustig & B. Kellner: Ztschr. f. Krebsforsch. *37*: 347 (1932).
67. E. Freund & B. Lustig: Wien. klin. Wchnschr. *46*: 1580 (1933).
68. A. F. Watson & E. Mellanby: Brit. J. Exper. Path. *11*: 311 (1930).
69. N. Waterman: Acta Cancrol. *2*: 375 (1936).
70. M. Oike: Jap. J. Obst. & Gynec. *14*: 421 (1931).
71. S. R. Benedict & K. Sugiura: J. Cancer Res. *11*: 311 (1930).
72. W. Caspari: Ztschr. f. Krebsforsch. *38*: 355 (1932).
73. W. Nakahara & E. Somekawa: Proc. imp. acad. *4*: 236 (1928); abstr. Cancer Rev. *4*: 476 (1929).
74. W. Caspari & F. Ottensooser: Ztschr. f. Krebsforsch. *38*: 351 (1932).

sponsible for this effect.[75] In a series of recent investigations, the carcinogenic action of benzpyrene, dibenzanthracene and methylcholanthrene was found increased in mice kept on a diet containing fifteen per cent of fat (lard, butter or vegetable oils). This enhancement of carcinogenesis took place in spite of the fact that the animals receiving this diet were in poor general health, but the stimulation seemed to be confined to surface tumors.[76, 77] A similar effect of fatty diets was found for tumors induced by means of ultra-violet radiation,[78] and, in further analysis of the stimulation of the formation of methylcholanthrene tumors in mice by foods rich in fat, fatty acids were held responsible for this effect. Heating of the fat to 300° C for one hour increased its tumor-promoting effect, but in general the stimulating action of alimentary fat was found less marked in rats than in mice, a fact which was thought to depend on differences in the skin textures and in the fat metabolism of these two species.[79] By placing mice on a diet rich in fat, the following effects on tumor development were observed. The incidence of spontaneous breast cancer was markedly increased, whereas the incidence of skin tumors following external application of carcinogens was decreased. The appearance of primary lung tumors was not affected by the diet, and the induction of sarcomas by means of injections of benzpyrene was either unaffected or slightly inhibited. The growth rate of tumors arising in animals fed the fat-rich diet was not significantly changed.[80] Thus it becomes apparent that different types of tumors are influenced in a different manner by the fat content of the food, and this may to some extent explain the conflicting statements which have been issued regarding the effects of this dietary constituent on neoplasia. As mentioned in chapter II, the ingestion of heated fat was claimed to result in the formation of gastro-intestinal neoplasms.[81] However, a recent study failed to support this contention; feeding of heated fats to rats produced a papillomatosis in the forestomach of the animals, but formation of malignant tumors was not observed. Since the addition of large doses of vitamin A prevented the development of papillomatosis in rats ingesting the heated fats, these substances were assumed to interfere in some manner with the vitamin A metabolism.[81a]

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[75] F. L. Haven: Am. J. Cancer *27*: 95 (1936).

[76] C. A. Baumann, H. P. Jacobi & H. P. Rusch: Am. J. Hyg. *30*: 1 (1939).

[77] H. P. Jacobi & C. A. Baumann: Am. J. Cancer *39*: 338 (1940).

[78] C. A. Baumann & H. P. Rusch: Am. J. Cancer *35*: 213 (1939).

[79] P. S. Lavik & C. A. Baumann: Cancer Research *1*: 181 (1941).

[80] A. Tannenbaum: Cancer Research *2*: 468 (1942).

Lipids contained in food have been generally assumed to promote the tumor growth. Actually, diets poor in lipids were found to inhibit transplanted tumors,[82] whereas a stimulation of tumor growth resulted from feeding animals with organ extracts of high lipid content,[83] and an enhancement of tar cancer was noted in mice fed egg yolks rich in phospholipids and cholesterol.[84] The high cholesterol concentration of food has been frequently observed to promote the growth of inoculated tumors [82, 85, 86] and to enhance the formation of tar cancer.[87, 88] Furthermore, the significant observation was made that an excessive cholesterol content in the diet increased the formation of metastases, but that, on the other hand, diets poor in cholesterol resulted in a lower number of takes of tumor inoculations.[85] Lanolin, which represents mainly oxycholesterol esters, was reported to enhance the development of tar cancer.[89] Yet the tumor promoting effect of alimentary sterol does by no means extend to all types of tumors, since diets rich in cholesterol failed to enhance the appearance of spontaneous mouse tumors,[90] and no influence in the formation of tumors in mice exposed to ultra-violet irradiation was noticed from the addition of two per cent of cholesterol to their diet ([78], cf. chapter II). Hence, it appears very doubtful whether the assumption was justified which connected an alleged increased cancer incidence among Basques and Irishmen with a high cholesterol content of the food used by these populations.[91] In general, the experimental evidence which indicates a promotion of the growth of certain types of animal neoplasms does not seem to warrant far-reaching conclusions as to a responsibility of alimentary sterols for the pathogenesis of human malignant tumors.

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[81] A. H. Roffo: Bol. inst. med. exper. para el estud. y trat. d. cáncer *15*: 539 (1938); Bull. Assoc. franç. p. l'étude du cancer *28*: 556 (1939).

[81a] S. Beck & P. R. Peacock: Brit. M. J. *2*: 81 (1941).

[82] S. Bernstein & H. Elias: Ztschr. f. Krebsforsch. *26*: 1 (1928).

[83] A. H. Roffo: Bol. inst. med. exper. para el estud. y trat. d. cáncer *7*: 539 (1930).

[84] L. Cirio: Pathologica *22*: 595 (1930).

[85] J. E. Sweet, E. P. Corson-White & G. J. Saxon: J. Biol. Chem. *21*: 309 (1915).

[86] G. Tesauro: Ztschr. f. Krebsforsch. *35*: 269 (1932).

[87] M. Borst: Ztschr. f. Krebsforsch. *21*: 334 (1924).

[88] W. Eber, F. Klinge & L. Wacker: Ztschr. f. Krebsforsch. *22*: 359 (1925).

A much less marked effect has been observed of lecithin feeding in animal experiments, since only slight, if any, growth inhibitions of transplanted tumors were reported.[82, 86] In view of the more pronounced impeding effect on the tumor process which has been reported of parenteral lecithin administration (cf. chapter II), it may be supposed that the peroral lecithin ingestion is less effective because of the enzymatic cleavage of this substance in the intestinal tract. Since digestion affects lecithin to a higher degree than cholesterol, it is understandable that the action of the latter substance is less dependent on the route of administration.

Inorganic food materials. Among the substances which are important for the maintenance of life, though lacking in caloric value, consideration may be given primarily to the consumption of water. Far-reaching limitation of the water ingestion in mice has been found to depress the yield of inoculated tumors, to lower the tumor growth rate, and to lessen the frequency of metastases.[92] Similar observations were made with transplanted rat tumors when the animals received a diet of low water content,[93] but it is most likely that these effects merely depended on the reduced caloric intake of the thirsting animals, and hence they must be regarded as a consequence of underfeeding.[9]

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[89] K. Lee, T. Fukuda & R. Kinoshita: J. Cancer Res. *8*: 127 (1924).
[90] T. B. Robertson: J. Biol. Chem. *37*: 443. (1919).
[91] A. H. Roffo: Ztschr. f. Krebsforsch. *38*: 369 (1932).

Little need be said of the effect of various inorganic ions on the tumor process since these relations have been detailed in chapter I. It may be repeated that only a few of the phenomena claimed to result from the administration of such substances have been verified beyond any doubt. A tumor promoting property of potassium salts—administered perorally or parenterally—has been made very probable, but the impeding action of calcium salts is much more problematic and the alleged antiblastic quality of magnesium must be regarded as wholly unfounded. Controversial opinions have been expressed regarding the most important mineral constituent of food, viz., sodium chloride; whereas some authors suspected this salt of stimulating neoplastic growth, and accordingly recommended its restriction in the cancer diet,[cf. 94] clinical observations indicated that regimes extremely poor in salt, such as have been used for dietary treatment of tuberculosis, exert a rather unfavorable influence on human neoplasia.[95, 96] The results of a small number of animal experiments, in which the effects of sodium chloride on tumors were studied, have not been very conclusive (cf. chapter I), and hence a more thorough analysis of this problem is desirable for elucidating the role of this dietary factor.

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[92] W. Caspari & F. Ottensooser: Ztschr. f. Krebsforsch. *32*: 394 (1931).

[93] R. Guastalla: Cancro *2*: 98 (1931); abstr. Ztschr. f. Krebsforsch. *35*: Ref. 29 (1932).

[94] A. Lorand: 1st Internat. Cancer Congress, Madrid *2*: 48 (1933).

[95] F. Blumenthal: 1st Internat. Cancer Congress, Madrid *1*: 793 (1933).

[96] E. Hesse: Deutsche med. Wchnschr. *61*: 797 (1935).

Another aspect of the possible significance of alimentary minerals for neoplasia is represented by their participation in the maintenance of the acid base equilibrium. In connection with this function of minerals and with theories on a general association of neoplasia with alkalosis (cf. chapter III), far reaching claims have been advanced, according to which a fundamental responsibility for the development of malignant tumors was assigned to the mineral content of food. In particular, a protein-poor and potassium-rich regime was claimed to offer favorable conditions for tumor development by inducing an alkalosis;[97, 98] it was reported that such diets led to the appearance of spontaneous tumors in mice[97] and, based on these observations, it was emphasized: "No cancer without alkalosis."[98] However, as shown in chapter III, this theory has been invalidated by a great number of experimental studies, and an authority in the field of the physiology and the pathology of nutrition has objected strongly to the view that diets producing alkalosis may be responsible for development of cancer.[99] The value of the aforementioned report on the formation of spontaneous tumors in animals fed an alkaline diet is very doubtful, since the number of experiments and controls appears insufficient and, in fact, a later study failed to observe any influence of alkaline nutrition on spontaneous tumor incidence, although such a regime enhanced the growth of transplanted tumors.[100]

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[97] O. L. E. de Raadt: Ztschr. f. Krebsforsch. *30*: 449 (1929); *32*: 596 (1930).

[98] L. von Gordon: Ztschr. f. Volksernährung *9*: 119 (1934).

[99] R. Berg: Ztschr. f. Volksernährung *9*: 277 (1934).

[100] A. Goldfeder: Ztschr. f. Krebsforsch. *38*: 121 (1932).

On the other hand, experiments in which tumor bearing mice were fed a diet of acid nature—consisting mainly of thymus, an organ rich in proteins and nucleic acids and poor in minerals—showed only slight and irregular retarding of the tumor growth.[101] In evaluating such studies it must be borne in mind that attempts to modify the hydrogen ion concentration of blood and tissues by means of dietary interferences always encounter great difficulties and hence appear to hold little promise.

Feeding of various organs. Apart from their known chemical composition, certain organs, or extracts prepared from them, appear to influence neoplastic growth when added to the diet of tumor bearing animals. As a result of a clinical observation of the extremely rare incidence of malignant tumors in the duodenum and the small intestines, feeding of these organs has been tried with animals bearing transplanted tumors, and this measure was reported actually to impede the tumor growth.[102] As will be mentioned in chapter VIII, a preparation recommended by Blumenthal and Jacobs [103] as a peroral organotherapy in cancer (I B 5) contained extracts of the small intestines, but the usefulness of this therapy has been far from established. An antiblastic effect has been demonstrated to result from the feeding of brain, or extracts of this organ,[104, 105, 105a] and this observation is the more remarkable since these substrates are particularly rich in lipids, which, as mentioned above, have been generally found to stimulate tumor growth. As will be detailed in chapter VIII, brain extracts of antiblastic qualities constituted a part of the organotherapy developed by Maisin and his collaborators for treatment of neoplasia.

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[101] P. Rondoni: Rend. Acad. Lincei *17*: No. 11 (1933).

[102] H. Kretzschmar: Ztschr. f. Krebsforsch. *28*: 154 (1928).

[103] E. Jacobs: Ztschr. f. Krebsforsch. *38*: 545 (1933).

[104] A. H. Roffo: Bol. inst. med. exper. para el estud. y trat. d. cáncer *5*: 170 (1926).

[105] H. Vassiliadis: Compt. rend. Soc. de biol. *118*: 1483 (1935).

[105a] C. Ronsse: Cancer, Bruxelles *12*: 319 (1935); abstr. Am. J. Cancer *29*: 369 (1937).

The development of tar cancer in mice was found enhanced by the feeding of liver or pancreas to the animals [106] and the tumor stimulating effect of liver feeding has been confirmed in numerous studies of various tumor bearing animals.[107-113] It is of practical significance that the tumor promoting agent contained in liver has been shown as not being identical with the antianemic liver principle.[114] However, it is interesting that both the liver and stomach extracts increased the susceptibility of rats to Walker sarcoma.[115] A fresh liver diet given to mice painted with benzpyrene caused only a slight acceleration of the papilloma formation, but slightly delayed the malignant transformation of these lesions.[116] An entirely different effect of liver feeding has been ascertained for a special instance of carcinogenesis, namely, the production of liver tumors in rats by means of feeding butter yellow (cf. chapter II); this carcinogenesis has been found to be prevented by a liver diet.[117, 118] This effect was recognized as being independent of vitamins or other known nutritional factors present in the liver,[118] and it was stressed that the

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106. A. F. Watson: Am. J. Cancer *19*: 389 (1933).
107. J. Maisin: Internat. Conf. Cancer, London, p. 17 (1928).
108. J. Maisin & M. A. François: Ann. de méd. *24*: 455 (1928).
109. H. D. Caylor: Proc. Staff Meet. Mayo Clin. *3*: 454 (1928).
110. H. D. Caylor, E. J. Baldes & F. C. Mann: Arch. Path. *11*: 854 (1931).
111. A. de Coulon & A. Ugo: abstr. Ztschr. f. Krebsforsch. *40*: Ref. 157 (1934).
112. A. Haddow: Am. J. Cancer *22*: 308 (1934).
113. J. Maisin & Y. Pourbaix: Am. J. Cancer *24*: 357 (1935).
114. J. Maisin: Ann. Surg. *93*: 180 (1931).
115. K. K. Rice: Am. J. Hyg. *25*: 11 (1937).
116. G. M. Bonser & L. M. Wainman: J. Path. & Bact. *50*: 548 (1940).
117. R. Kinosita: Tr. Soc. jap. path. *27*: 665 (1937).
118. W. Nakahara, K. Mori & Fujiwara: Gann *32*: 465 (1938); *33*: 406 (1939).

impeding action of feeding of liver was limited to the tumorigenic stimulus of butter yellow, but did not extend to benzpyrene or methylcholanthrene.[119] A lesser degree of inhibition of tumor formation in rats by means of butter yellow was also observed from feeding of kidney, and it was noticed that the cysteine content of liver or kidney is not responsible for their antiblastic activity.[120] No definite evaluation can as yet be given for these influences on neoplastic processes which have resulted from the administration of certain organs in the food, nor can it be explained why these effects differ in various forms of neoplasia. It may tentatively be assumed that they depend on some unknown factors, perhaps of vitamin or enzyme nature, and these hypothetical agents may interfere with tumor development and tumor growth via metabolic mechanisms. Furthermore, it may be pointed out that, as mentioned in chapter IX, the presence of growth inhibiting principles, and of growth stimulating principles, in the same biological substrate has frequently been demonstrated, and hence it may be assumed that according to the methods of preparation and the special carcinogenic mechanism concerned, the action of one or the other category of substances may predominate. In any event, further work on these problems will be necessary before any definite conclusions can be drawn from the observations thus far made.

Condiments. On first sight it may appear very improbable that condiments in food may influence pathological processes, but it has been demonstrated that some of these substances are capable of interfering with bacterial growth,[121] and in this manner they may counteract processes of intestinal fermentation and putrefaction. Since these processes have been considered to favor the development of neoplasia, the liberal use of garlic as an auxiliary measure for the treatment of cancer has been recommended.[122] In this connection reference has been made to a purportedly rare incidence of malignant tumors in countries where garlic is used in great quantities (Southern Italy, Greece). Although such assumptions are mainly speculative, some concrete evidence has been presented by animal experiments, inasmuch as the prophylactic feeding of fresh garlic, but not of commercial preparations, to mice decreased the number of takes of Ehrlich carcinomas.[123, 124] The life of tumor bearing animals on the garlic diet was prolonged, but the growth of existing tumors was only slightly and temporarily impeded.[124] Allyl compounds present in garlic were regarded as representing the active agent.[2] In this connection it may be mentioned that hypothetically an antiblastic activity was ascribed to allyl compounds, because of their ability to act as oxygen acceptors and, in this manner, to prevent a dehydrogenation of sterols or bile acids into substances of carcinogenic activity ([125,] cf. chapter VI). Yet it must be remarked that the conversion of bile acids into carcinogenic compounds in the body has not been proved, nor has an antiblastic effect of allyl compounds been convincingly established. As discussed in chapters II and IX, aldehydes, principally heptyl aldehyde, have been recognized as being responsible for a remarkable effect in depressing the incidence of spontaneous tumors in mice, when these animals were fed pimento oil [126] or gaultheria (wintergreen) oil;[127] details of this phenomenon and its interpretation are to be found in the chapters mentioned.

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[119] K. Mori & W. Nakahara: Gann *34*: 48 (1940).

[120] K. Mori: Gann *35*: 86; 106; 121 (1941).

[121] W. Kolle, K. Lauberheimer & H. Vollmar: Wissenschaftl. Woche, Frankfurt a, M., Vol. *3*: 1 (1935).

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[122] A. Lorand: Med. Klinik *30*: 1030 (1934).

[123] W. Caspari: Ztschr. f. Krebsforsch. *43*: 255 (1936).

[124] L. Makowski: Ztschr. f. Immunitätsforsch. u. exper. Ther. *89*: 423 (1936).

[125] A. Müller: Wien. klin. Wchnschr. *50*: 1614 (1937).

[126] L. C. Strong: Am. J. Cancer *25*: 607 (1935).

2. *Vitamins.*

The progressing research on vitamins has been accompanied by numerous attempts to establish basic connections between these potent biological agents and tumor pathology, since it is conceivable that a lack or an excess of these vital substances may be responsible for anomalies of metabolism and growth exhibited by malignant cells. In fact, one of the founders of vitaminology, C. Funk,[128] was inclined to see an avitaminosis in neoplasia, but unfortunately the vast number of investigations in this field by no means substantiated this contention, and the problem appears to be highly complex. For evaluating it properly, it appears necessary to bear in mind the following factors: on the one hand, most vitamins are indispensable to normal growth or for the maintenance of normal metabolism and, accordingly, a deficiency will impair the vitality of neoplastic tissues in a manner similar to the effect on normal organs. On the other hand, excessive administration of one vitamin or the other, or disturbances of the vitamin equilibrium, are liable to interfere with various metabolic mechanisms and hence may influence the neoplastic process in one of various ways. At the same time it must be stressed that, as is frequently the case in tumor pathology, the effects of hypervitaminosis or hypovitaminosis may be entirely different as to the initiation of neoplasia and the growth of existing tumors.

In the following presentation, analytical data regarding the presence of the individual vitamins in neoplasms and in the body of tumor hosts will be discussed and the experimental findings, which have been derived from studies on the effect of vitamins, or their lack, on the tumor process will then be surveyed. Prior to this systematic survey, a number of investigations must be considered, since in part they were performed at a time when insufficient knowledge was available as to the action of individual vitamins. Accordingly, this work concerns the influence of vitamins as a whole, that is, the effect of lack of vitamins in the food, or of excessive vitamin intake. It may be taken as generally agreed upon that avitaminosis considerably impedes or nullifies the success of tumor inoculation.[40, 85, 129-133] Some contradictory statements implying a normal development of transplanted tumors in avitaminotic animals [10] may have been due to an incomplete exclusion of vitamins from the diet. It has, however, been ascertained beyond any doubt that diets poor in vitamins may fail to affect the growth of already developed tumors.[134, 135] On the other hand, hypervitaminosis has been reported to increase the frequency of recurrences following excision of inoculated tumors and also to facilitate the growth of tumors transplanted to animals of a foreign species (heterotransplantation), whereas avitaminosis resulted in the opposite effect (cf. chapter VIII). Furthermore an excessive administration of vitamins appeared to counteract one special instance of tumor inhibition, that is, in pregnant and lactating animals (cf. chapter VII). Accordingly, it was assumed that the inhibiting effect of pregnancy was due to the high vitamin requirement of the embryo and a consequent vitamin

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[127] L. C. Strong: Am. J. Cancer *20*: 387 (1934); *23*: 297; *25*: 797 (1935); Am. J. M. Sc. *192*: 546 (1936).

[128] C. Funk: Die Vitamine, München 1924.

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129. S. R. Benedict & A. H. Rahe: J. Cancer Res. *1*: 159 (1917).
130. F. Ludwig: Ztschr. f. Krebsforsch. *23*: 2 (1925).
131. P. Geréb: Ztschr. f. Krebsforsch. *36*: 245 (1932).
132. E. Fränkel & P. Geréb: Ztschr. f. Krebsforsch. *38*: 524 (1933); *39*: 93; 97; 101 (1933).
133. I. Miscenko & M. Fomenko: Vopr. Oncol. *7*: 236 (1935); abstr. Acta Cancrol. *2*: Ref. 19 (1936).
134. G. Joannovics: Wien. klin. Wchnschr. *29*: 345 (1916).
135. F. Ludwig: Schweiz. med. Wchnschr. *54*: 232 (1924).

deprivation of the tumor.[131, 132] It must, however, be remarked that the last named findings have not undergone re-examination by other investigators, and the tendency to relate all variations of tumor growth solely to vitamins must be considered an over-simplification of the problem. Conflicting reports have been announced as to the influence of vitamins on the development of tar cancer. In one study an inhibition of carcinogenesis was ascribed to avitaminosis and a stimulation to hypervitaminosis,[136] yet just the opposite results were obtained by another investigator who reported a failure to induce tar cancer in mice fed a diet rich in vitamins.[137] Moreover, he announced that a diet rich in vitamins caused general improvements in six cancer patients and prolonged their lives.[137] The administration of large quantities of water-soluble vitamins (B_1, B_2, C) has been found to affect only slightly carcinogenesis induced in mice by means of methylcholanthrene; that is, only animals, in which the increased vitamin intake resulted in particularly good health, showed a more rapid development and growth of malignant tumors as compared with control animals.[138] This observation may be taken as a confirmation of the opinion previously stated, viz., that vitamins enhance neoplastic processes in the same manner as they benefit the general vitality of the body. Additional corroboration of this view may be found in studies in which the direct approach of observing cellular growth in tissue cultures was employed; a strong growth inhibition of both normal fibroblasts and of neoplastic cells was noted when plasma of avitaminotic animals was used as the medium.[139] In continued work it was ascertained that a deficiency in vitamins A and B_1 of the plasma was particularly responsible for the suppression of the growth of the tissue cultures, whereas an excess of these vitamins stimulated the growth of normal and neoplastic cells in vitro.[140]

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[136] P. Geréb: Tumori n. s. *10*: 177 (1936).

[137] J. R. Davidson: Canad. M. A. J. *38*: 529 (1938).

[138] H. J. Lauber, H. Schocke & Th. Bersin: München. med. Wchnschr. *85*: 1741 (1938).

[139] T. Gordonoff & F. Ludwig: Ztschr. f. Vitaminforsch. *4*: 213 (1935).

Particular consideration must be given to the effects of vitamin deficiency in connection with alterations of the gastro-intestinal tract, which may be regarded as precancerous lesions. The findings of older studies on this problem have recently been critically reviewed, as well as supplemented by new experimental data.[141-148] Various forms of malnutrition, characterized by vitamin deficiencies, have been found to produce papillomas and ulceropapillomas in the forestomach of rats. These lesions were described as benign, they healed after the animals were restored to a normal diet, and were not believed to be caused by lack of any of the more widely known vitamins (A, B_1, B_2, C, D).[141] Other workers observed the formation of gastric lesions (papillomas of the forestomach) in rats subjected to fasting and partial inanition; the authors of this study, however, did not consider the lack of any particular foodstuff to be responsible for the occurrence of these lesions, since addition of either carbohydrates, fats, or proteins to the diet prevented or cured the papillomas.[148] These findings from animal experimentation met a significant counterpart in clinical observations, demonstraing a connection between dietary deficiencies and the development of chronic gastritis. For certain forms of the latter pathology it has been made highly probable that they represent precancerous lesions and, although it was by no means claimed that all forms of gastric cancer are the results of such nutritional disturbances, the phenomena described are

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140. T. Gordonoff & F. Ludwig: Ztschr. f. Krebsforsch. *46*: 73 (1937).
141. A. Brunschwig & K. A. Rasmussen: Cancer Research *1*: 371 (1941).
142. A. J. Klein & W. L. Palmer: J. Nat. Cancer Inst. *1*: 559 (1941).
143. H. P. Morris & S. W. Lippincott: J. Nat. Cancer Inst. *2*: 459 (1942).

of great interest and may be expected to form a basis for continued investigations.[144]

Vitamin A. This fat-soluble vitamin appears to be involved in a number of physiological processes which are significant in connection with the tumor problem. It is a familiar fact that lack of vitamin A is followed by severe disturbances of the epithelial tissue, expressed in keratinization and metaplasia, observations which induced one author to designate vitamin A as the "epithelium-protective vitamin." [145] This capacity of maintaining the normal epithelial structure appears to be linked with a regulating function of the lipid metabolism exerted by the vitamin, particularly as to the deposit of cholesterol in the tissues.[146] An involvement of vitamin A in the oxidative metabolism has been assumed on the basis of the observation that healthy persons reacted to the administration of this vitamin with an increase of the carbon/nitrogen ratio in the urine, a finding which was taken as an expression of an inhibition of oxidation.[147] However, in the absence of further studies on this phenomenon, it cannot be as yet definitely evaluated.

Analytical data on the presence of vitamin A in malignant tumors have been made available by some earlier investigations which, however, must be cautiously interpreted, since it has been recognized that the color reaction with antimony chloride employed for the vitamin A estimation is by no means specific. A very low vitamin A concentration was determined for one melanosarcoma of a horse,[148] yet because of the isolated nature of this finding it possesses only a limited value. A greater number of human neoplasms were tested by another worker who found vitamin A present in eleven out of twelve carcinomas and in two out of three sarcomas. The vitamin concentration of the tumors was reported to have been proportional to their growth intensity.[149] Yet, in addition to the unreliability of the color reaction used for the vitamin determination, the results mentioned are of limited value because they failed to indicate whether the vitamin was actually present in the tumor cells, or whether it was merely contained in the tumor stroma. For these reasons, a greater significance must be attributed to extensive studies of recent date in which the vitamin A was assayed by a direct histochemical method, viz., by fluorescence microscopy of normal and of neoplastic tissues. In this manner, it was first ascertained that vitamin A and carotene are stored principally in the Kupffer cells and the epithelial cells of the liver, in endothelial cells of the lung, kidney and liver, and that it is always connected with the presence of fat ("vital stain of lipids") ; tumor cells were found to contain vitamin A only as an exception.[150] The latter findings were verified on a large scale by testing 219 human malignant tumors. Whereas the vitamin was frequently found present in interstitial cells and fat, the tumor cells proper were found free of vitamin A with the exception of but seventy-six neoplasms, and the vitamin content of these tumors was shown to depend on the presence of the vitamin in the parent tissue from which the tumors were derived. It may be mentioned here that on the basis of these histological observations and from a review of the literature the author concluded that vitamin A has no bearing on the tumor etiology.[151] The results of these clinical studies are in satisfactory agreement with those of animal experimentation. Flexner-Jobling rat carcinomas and rat sarcomas were found free of vitamin A[152] nor could the vitamin be detected in various transplanted rat and rabbit tumors.[153] It is significant that these relations were also not changed after the administration of large vitamin doses to the animals.[152, 153] Regarding the ascites carcinomas of mice it was reported that the cells in the first stages of tumor development exhibited an increase of vitamin A, but that the vitamin gradually diminished and was no longer present in the last stages of the tumor process.[154]

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[144] C. P. Rhoads: J. Nat. Cancer Inst. *1*: 511 (1941).

[145] W. von Drigalski: Ztschr. f. Vitaminforsch. *3*: 37 (1934).

[146] J. A. Collazo, I. Torres & Sanchez-Rodriguez: Klin. Wchnschr. *13*: 1678 (1934).

[147] F. Lauersen, K. Voit & H. Wendt: Klin. Wchnschr. *16*: 1047 (1937).

[148] S. V. Gudjonsson & G. Oppenheim; Ztschr, f. Krebsforsch. *33*: 155 (1931).

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[149] E. Vogt: Med. Klin. *28*: 1344 (1932).

[150] H. Popper: J. Mt. Sinai Hosp. N. Y. *7*: 119 (1940) ; Arch. Path *31*: 766 (1941) ; *32*: 11 (1941).

[151] H. Popper & A. B. Ragins: Arch. Path. *32*: 258 (1941).

A great deal of work has been devoted to quantitative determinations of the vitamin in the body of tumor hosts. A decrease of the vitamin A concentration in the blood of cancer patients has been announced. This alteration was found to run parallel to the progress of the disease, but to be independent of the urinary vitamin A excretion.[155] A precise experimental analysis of the plasma vitamin A level in neoplasia has been performed recently, with particular consideration of gastro-intestinal cancer. The following significant results were obtained: the vitamin values found in the plasma of patients with gastro-intestinal cancer were reduced in eighty-six per cent of the cases examined. Following successful resection of the tumors, the plasma vitamin level was frequently found normal and a lowered vitamin A content was also determined in plasma of patients suffering from other malignant tumors, principally bone sarcomas or pancreatic carcinomas; normal values were, however, observed in patients with mammary or with oral cancer. Analyses of these results made it improbable that the reduced vitamin content of the plasma was due to dietary deficiencies or to mal-absorption of the vitamin; furthermore, it is of great interest that the administration of vitamin A markedly raised the plasma vitamin level in patients with a low vitamin level who were suffering from disorders other than gastro-intestinal cancer, whereas in the latter cases the vitamin administration failed to bring about this effect. It was assumed that in the latter cases the reduced vitamin A concentration in the plasma is caused by a hepatic dysfunction, and to what extent this view has found experimental support will be discussed later. Finally, it was shown that in patients with gastro-intestinal cancer an increase of the diminished plasma values of vitamin A could be achieved by administration of yeast, of lipocaic, or of choline chloride, an effect which was believed to depend, perhaps, on the lipotropic activity of the substances named.[156]

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[152] A. Goerner & M. M. Goerner: J. Biol. Chem. *123*: 57 (1938).
[153] Y. Huzita: Jap. J. Obst. & Gynec. *22*: 45 (1939).
[154] P. G. Seeger: Arch. f. exper. Zellforsch. *24*: 59 (1940).
[155] F. Grant: Ztschr. f. klin. Med. *133*: 168 (1937).

As to other organs of tumor hosts, an older report dealt with the pituitary gland; it was observed that the glands of cancer patients who had ultimately died in a state of extreme cachexia showed low values of vitamin A, whereas an appreciable vitamin increase was found in patients who had succumbed rapidly to the disease.[157] Hence these results do not appear to have a direct bearing on the tumor process; it may rather be assumed that the vitamin depletion of the pituitaries in the cancer patients dying of cachexia was merely a consequence of a general inanition, and this is the more probable since low vitamin concentrations were also found in the pituitaries of patients deceased of sepsis.[157] A number of studies have been concerned with the vitamin A content of the livers of tumor hosts, a problem of great interest since this organ is of primary importance for the formation, storage, and distribution of this vitamin.[158] The results of one

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156. J. C. Abels, A. T. Gorham, G. T. Pack & C. P. Rhoads: J. Clin. Investigation *20*: 749 (1941).
157. E. Vogt: Med. Klinik *29*: 1734 (1933).
158. T. Moore: Biochem. J. *25*: 275 (1931).

study indicated a diminution to about one half of the normal value of the vitamin A concentration in hepatic tissue of persons deceased from cancer, but it was stressed that a similar reduction of the hepatic vitamin A values also occurred in other diseases, in some of which even lower values were determined than in livers of cancerous patients.[159] It may have been due to the selection of cases that the results of this study have been confirmed neither by clinical investigations nor by animal experiments. As far as the latter are concerned, reference is made to findings of normal vitamin A concentrations in the liver of rats with transplanted carcinomas and sarcomas,[152] and to a report according to which the vitamin A content in the livers of rats with spontaneous tumors exceeded the normal values.[160] Furthermore, the liver tissues of fourteen patients with gastro-intestinal cancers were shown to exhibit vitamin A concentrations which only in two instances were below the normal range.[161] Accordingly, it was concluded that the aforementioned observations of low vitamin levels in the plasma of these patients cannot be charged to an impaired capacity of the liver to store the vitamin, but it was instead tentatively assumed that in these patients a deficiency exists of an agent which mobilizes the vitamin from the liver.[161] Attention has been called to the effect of carcinogens on the vitamin A content of livers, but this problem still awaits a final clarification. For, on the one hand, a marked decrease of vitamin A was observed in the livers of rats and rabbits which received intraperitoneal injections of dibenzanthracene. In these animals, carotene was not converted into vitamin A, and injected vitamin A was apparently not stored in the liver.[162, 163] Similarly, rats fed aminoazotoluene showed reduced hepatic vitamin values, and the hepatic tumors arising were found free of vitamin A, whereas the surrounding normal liver tissue was capable of storing the vitamin.[164] Yet, on the other hand, it was observed that a marked vitamin A depletion occurring in the liver of rats injected intraperitoneally or subcutaneously with dibenzanthracene resulted, only to a lesser degree, from injections of carcinogens of higher potency, such as methylcholanthrene or benzpyrene, but feeding of butter yellow was entirely ineffective. For this reason, the vitamin A depletion of the liver was not regarded as being directly connected with the carcinogenic activity of the substances named, and the aforementioned data of high vitamin A values in livers of rats with spontaneous tumors was taken as proving that a hepatic vitamin A depletion is not a necessary condition for tumor formation.[160] Likewise, another author obtained results which make it improbable that a primary connection exists between the carcinogenic property of compounds and their effect on the vitamin A content of the liver. Intraperitoneal injections of methylcholanthrene into rats caused a marked reduction of the hepatic vitamin A concentration; injections of benzpyrene or of phenanthrene had a similar, but weaker, effect. In mice, however, intraperitoneal injections of methylcholanthrene or of phenanthrene did not change the vitamin A content of the liver.[165] It is apparent from these observations that different animal species show a different response, and that administration of noncarcinogenic compounds may also affect the vitamin storage in the liver.

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[159] T. Moore: Biochem. J. *31*: 155 (1937).

[160] C. A. Baumann, E. G. Foster & P. S. Lavik: J. Nutrition *21*: 431 (1941).

[161] J. C. Abels, A. T. Gorham, G. T. Pack & C. P. Rhoads: Proc. Soc. Exper. Biol. & Med. *48*: 488 (1941).

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[162] A. Goerner: J. Biol. Chem. *122*: 529 (1938).
[163] A. Goerner & M. M. Goerner: Am. J. Cancer *37*: 518 (1939).
[164] A. Goerner & M. M. Goerner: J. Biol. Chem. *128*: 559 (1939).
[165] C. Carruthers: Cancer Research *2*: 168 (1942).

In general, no characteristic significance has been assigned to the excretion of vitamin A which has been frequently observed in the urines of cancer patients.[166-170] Although normally no vitamin A is excreted in the urine, it may be frequently encountered in the urines of patients with tuberculosis, severe infections, or with malignant tumors, but the fact that this phenomenon is not restricted to neoplasia made its secondary nature plausible, and the presence of a hepatic dysfunction was considered as probably responsible for the occurrence of the vitamin in the urines.[169] Actually, the urinary vitamin A excretion was found to be most frequently associated with liver cirrhosis or with liver metastases,[171] and in general the disturbances of the vitamin A metabolism which may be observed in later stages of neoplasia were believed to be due to secondary factors such as cachexia and infection.[172]

Although the analytical data do not appear to indicate a primary participation of vitamin A in carcinogenesis, experimental findings have been announced from which it has been inferred that a vitamin A deficiency may be an important factor in tumor formation. Thus rats receiving a diet free of vitamin A were reported to develop epithelial metaplasia and proliferative reactions, and, in some instances, eventually intestinal tumors.[173, 174] Similar effects were observed by other investigators,[175] and in particular a hyper-

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166. R. Boller: Ztschr. f. klin. Med. *130*: 163 (1936).
167. R. Boller & O. Brunner: Klin. Wchnschr. *15*: 1106 (1936).
168. R. Boller, O. Brunner & E. Bodaty: Wien. Arch. f. inn. Med. *31*: 1 (1937).
169. E. Schneider & H. Weigand: Klin. Wchnschr. *16*: 441 (1937).
170. T. Lindquist: Acta med. Scandinav. Suppl. *97* (1938).
171. R. Boller, O. Brunner & E. Bodaty: Klin. Wchnschr. *16*: 861 (1937).
172. E. Schneider: Arch. f. klin. Chir. *190*: 397 (1937).
173. T. Saiki & Y. Fujimaki: Deutsche med. Wchnschr. *53*: 517 (1927).
174. Y. Fujimaki: J. Cancer Res. *10*: 469 (1926); Gann *21*: 48 (1927).
175. H. Goldblatt & M. Benischek: J. Exper. Med. *46*: 699 (1927).

keratosis of the mammary gland resulting from A-avitaminosis was regarded as a factor favoring tumor development.[176] More recently it has been announced that a vitamin A-deficient diet administered to rats for eight to nine months caused metaplastic skin lesions which, in a small number of animals, were transformed into skin cancer.[177, 178] Yet, in evaluating the latter studies, it must be pointed out that the number of animals used was comparatively small and, regarding the earlier communications,[173-175] it must be stressed that the malignant nature of the lesions arising in A-avitaminotic animals has been strongly questioned by other investigators. This critical attitude appears justified, particularly in consideration of the failure of other workers to induce tumor formation in animals by means of vitamin-A-deprivation,[179] and likewise conclusions drawn from recent studies make it very improbable that a vitamin A deficiency by itself regularly induces neoplasms in animal experiments.[141, 180, 181] Proliferations of the stomach mucosa occurring in rats with A-avitaminosis were only rarely found to result in malignant tumors.[181] However, in the writers' opinion, a proper evaluation of the earlier studies must take into account the fact that the animals received a diet which was poor in vitamin A, but rich in vitamin B; hence the effects observed may have been due to a disturbance of the vitamin equilibrium, and this assumption appears supported by experimental work which demonstrated the action of dietary vitamin A lack as depending on the presence or absence of other vitamins,[182] as will be described in detail later. In addition, it may be suggested that quantitative relations determine to some extent the action of the vitamins, since it was found that both a deficiency and an excess of vitamin A stimulated tumor formation in gall-bladders into which stones had been inserted.[183] The circumstances enumerated may also represent a partial explanation for somewhat divergent statements which have been made as to the effect of vitamin A on the growth of transplanted tumors. Several workers observed a growth inhibition of transplanted tumors in animals receiving large vitamin A doses.[8, 133, 184, 185] The latent period of tumor development following inoculation was found to be prolonged as was also the length of the life of these animals, whereas the frequency of metastases was reduced.[186] Conversely, vitamin A withdrawal promoted the growth of transplanted tumors,[187] and extensive studies with a transplanted rat sarcoma yielded the following results: vitamin A deficiency impeded the tumor growth, a moderate A-hypervitaminosis caused a slight growth stimulation, and a strong A-hypervitaminosis again retarded the tumor growth and increased the radiosensitivity of the tumors.[188] An interesting contribution to the subject under discussion was furnished by the finding that a diet rich in vitamin A only slightly impeded the growth of transplanted tumors, but a strong tumor inhibition took place when a diet poor in vitamin A was subsequently replaced by a diet rich in vitamin A,[189] an observation which supports the assumption that disturbances of the vitamin balance may be very effective. It cannot be definitely decided, however, to what extent these factors, quantitative relations, or the types of tumors tested, were responsible for a number of conflicting results. In complete contrast to the aforementioned statements, a growth stimulation of transplanted tumors was reported to result from vitamin A administration, whereas lack of the vitamin impeded the growth.[190] Other workers failed to notice any influence of large doses of vitamin A on Walker rat carcinosarcoma 256 [191] or on transplanted rat tumors, or on tumors induced by dibenzanthracene.[152] By using tissue cultures as the test object, it was observed that the addition of vitamin A promoted the growth of normal as well as of neoplastic cells;[189] but this finding was later amended in that only small vitamin A concentrations stimulated the growth of cancerous cells in vitro, whereas large vitamin concentrations impeded the growth.[192] These results may perhaps be interpreted as indicating that moderate vitamin quantities are required for all growth processes, but that larger amounts may interfere with the functions of tumor cells by affecting metabolic processes.

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[176] E. Harde: Compt. rend. Soc. de biol. *116*: 999 (1934).

[177] A. Pallotti: Bull. d. sc. med. *107*: 526 (1935); abstr. Ztschr. f. Krebsforsch. *44*: Ref. 70 (1936).

[178] F. D. Rezzesi: abstr. Ztschr. f. Krebsforsch. *44*: Ref. 211 (1936).

[179] K. Sugiura & S. R. Benedict: J. Cancer Res. *14*: 306 (1930).

[180] Th. Hart de Ruyter: Acta brev. neerl. physiol. *4*: 122 (1934).

[181] L. S. Fridericia, S. Gudjonsson, Bj. Vimtrup, S. Clemmesen & J. Clemmesen: Am. J. Cancer *39*: 61 (1940).

[182] Rh. Erdmann: Arch. f. exper. Zellforsch. *3*: 368 (1927).

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[183] M. Matsudairo: abstr. Ztschr. f. Krebsforsch. *40*: Ref. 95 (1934).
[184] W. Caspari & F. Ottensooser: Ztschr. f. Krebsforsch. *30*: 1 (1929).
[185] B. Lustig & H. Wachtel: Biochem. Ztschr. *271*: 345; 357 (1934).
[186] V. Bisceglie: Tumori *17*: 145 (1931).
[187] G. Villara: Riv. pat. sper. *11*: 128 (1933).
[188] Y. Huzita: Jap. J. Obst. & Gynec. *21*: 370; 375; 378 (1938).
[189] C. Dittmar: Ztschr. f. Krebsforsch. *44*: 73 (1936).

No complete agreement has been reached as to the influence of vitamin A on carcinogenesis induced by chemical compounds. A marked inhibition of tar cancer formation has been seen in rabbits receiving large quantities of vitamin A.[193] Injections of vitamin A or of carotene were reported to impede the tumor production in mice by means of benzpyrene [194] and, likewise, vitamin A was found to impede the formation of hepatomas in rats fed aminoazotoluene.[195]

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190. Y. Ihara: Mitt. Path. (Sendai) *7*: 133; 151 (1931).
191. B. Sure, K. S. Buchanan & H. S. Thatcher: Am. J. Cancer *27*: 84 (1936).
192. H. Vollmar: Arch. f. exper. Zellforsch. *23*: 42 (1939).
193. M. Okishio: Tr. Jap. Path. Soc. *23*: 632 (1933).
194. C. Dittmar: Ztschr. f. Krebsforsch. *50*: 20 (1940).
195. F. Ishiwara & K. Saga: Gann *33*: 240 (1939).

But in contrast to these statements, it was reported that the formation of tumors in rats treated with benzpyrene was not influenced by a high or low vitamin A content of the diet.[196] It must be pointed out that no final evaluation of this problem can be given, since different effects may result, on the one hand, from injected or perorally administered vitamin, and, on the other hand, the influence of the vitamin may vary according to the animal species tested and the carcinogen employed.

Little need be said of therapeutic experiments with vitamin A in human neoplasia, this measure having so far proved of no definite value. Injections and external applications of vitamin A were reported to cause some diminution in the size of skin cancers, but complete cures of the tumors were never achieved, and the treatment seemed to have a rather unfavorable effect on metastatic lymph nodes.[197] Another author found vitamin A injections to cause only a slight and temporary growth inhibition in a few cancer cases treated, although the patients showed some subjective improvement and gains in weight.[166] In this connection, reference may be made to some correlations which have been established as existing between vitamin A and its precursor, viz., carotene, and the reticulo-endothelial system, a problem which is more fully considered in chapter VIII. On one hand, histological examinations revealed a severe damage of the reticulo-endothelial structures in animals treated with excessive doses of vitamin A,[198, 199] and a decreased storage of vitamin A in the liver was observed following interference which damaged the reticulo-endothelial function.[200, 201] Likewise, the study of three cases of healed massive liver necrosis in human beings indicated that vitamin A is stored principally in the Kupffer cells of the liver.[201a, cf. 150, 151] On the other hand, moderate amounts of carotene or vitamin A were found to stimulate the reticulo-endothelial activity, as measured by the Congo Red test ([202, 203,] cf. chapter VIII). Although no definite evidence is available, it seems conceivable that this interdependence of vitamin A and the reticulo-endothelial system may be responsible both for the deviations of the vitamin metabolism observed in neoplasia as well as for the influence of the vitamin on the tumor process. In the latter respect, this could serve as an explanation for some of the divergent effects enumerated, inasmuch as variations in the dosage of vitamin A administered might bring about either a stimulation or an impairment of the reticulo-endothelial function.

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[196] P. R. Howe, M. D. Elliot & M. J. Shear: Am. J. Path. *16*: 295 (1940).

[197] B. Lustig & H. Wachtel: Ztschr. f. Krebsforsch. *44*: 53 (1936).

[198] W. v. Drigalski & W. Laubmann: Klin. Wchnschr. *12*: 1171 (1933).

[199] Th. Moll, O. Dalmer, P. v. Dobeneck, G. Domagk & F. Laquer: Arch. f. exper. Path. u. Pharmakol. *170*: 176 (1933).

[200] F. Lasch & D. Roller: Klin. Wchnschr. *15*: 1636 (1936).

[201] H. Wendt & D. König: Klin. Wchnschr. *16*: 1253 (1937).

Some supplementary data may be given regarding the pro-vitamin A, viz., carotene. As far as analytical data were concerned, no deviations from the normal were found in the carotene content of malignant tumors, or of the blood of tumor hosts.[204] Experimentally, the administration of carotene failed to influence the formation of tar cancer in mice;[205] injections of carotene directly into Ehrlich mouse carcinomas caused a liquefaction of the tumors,[206] but this manner of administration cannot be regarded as being adequate for proving an antiblastic quality of carotene. Peroral ingestion of carotene in large quantities was followed by an impeded growth of transplanted mouse carcinomas, but this phenomenon was irregular, a fact probably due to variations in the carotene absorption.[207] Another investigator, however, re-

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[201a] A. J. Cox: Proc. Soc. Exper. Biol. & Med. *47*: 333 (1941).

[202] K. Stern & R. Willheim: Ztschr. f. d. ges. exper. Med. *97*: 354 (1935).

[203] Goyanes; quot. loc. cit. 146.

[204] H. M. Dyer & J. H. Roe: Am. J. Cancer *18*: 888 (1933).

[205] B. Bloch: Schweiz. med. Wchnschr. *57*: 1218 (1927).

[206] J. Kabelik: Compt. rend. Soc. de biol. *110*: 394 (1932).

[207] C. Kuh: Yale J. Biol. & Med. *5*: 123 (1932).

ported that daily feeding of twenty γ of carotene to rats with Jensen sarcoma stimulated the tumor growth; tumors of treated animals reached about double the size of tumors in control animals.[208] As will be described in chapter VI, carotene has been found by virtue of its oxygen avidity to influence markedly processes of oxidation and of glycolysis. This circumstance, together with the previously mentioned effect of carotene on the reticulo-endothelial system, may account for the influences on the tumor process which were reported to result from carotene administration. Subcutaneous injections of colloidal carotene solutions impeded the development of inoculated mouse and rat tumors; these effects, however, were not regular, probably because of variations in the particle size of the carotene solutions and also because of their instability, resulting readily in oxy-carotene.[202]

Vitamin B complex. The interpretation of a number of older studies meets with some difficulties, since the scientific progress made within the last few years revealed the vitamin B to consist of several individual vitamins different in their chemical composition and their biological action. Accordingly, both the analytical and the experimental studies carried out prior to the precise knowledge of the nature of the vitamin B complex must be evaluated with some reserve, and they are in need of renewed investigations performed by modern methods.

Analytical data have been furnished for malignant tumors by means of bio-assay of the antineuritic factor, i.e., B_1 or thiamine; rat sarcomas and carcinomas,[209, 210] fowl sarcomas [211] as well as mouse tumors and human neoplasms [212]

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208. H. v. Euler & G. Schmidt: Ztschr. f. physiol. Chem. *223*: 215 (1934).
209. J. C. Drummond: Biochem. J. *12*: 25 (1918).
210. W. Nakahara & E. Somekawa: Proc. imp. acad. *6*: 116 (1930); abstr. Ztschr. f. Krebsforsch. *33*: Ref. 24 (1931).
211. W. Nakahara & E. Somekawa: Proc. imp. acad. *4*: 440 (1928); abstr. Cancer Rev. *4*: 475 (1929).
212. H. Jackson, Jr. & C. J. Krantz: J. Clin. Investigation *6*: 508 (1928/29).

have been reported to exhibit a reduced content of this vitamin, whereas the values determined for the body of tumor-bearing animals did not deviate from the normal.[213] Some studies, however, led to divergent results, inasmuch as a high vitamin B_1 concentration was reported for Jensen rat sarcomas,[214] and the vitamin B_1 content of Rous sarcomas was found to parallel the growth rate of the tumors.[215] More recently, the thiamine level of sera of cancer patients measured by chemical methods was found to be reduced, and a decrease of the urinary excretion of this vitamin was also frequently observed in neoplasia.[216] Interesting data were recently reported regarding the thiamine content of white blood corpuscles. Normal leucocytes were found to contain a pyrimidine compound, regarded as a metabolite of thiamine, which accelerates the yeast fermentation (PAYF). It was demonstrated that white blood cells of leukemic patients contain more thiamine, but less of the PAYF-factor, than normal leucocytes; this finding was interpreted as indicating a faulty utilization of thiamine in this disease. It is noteworthy that similar deviations in the content of thiamine and the PAYF-factor seemed to be exhibited by leucocytes of patients with gastro-intestinal cancer, although a systematic analysis of these cases was not undertaken.[217]

More uniform results have been obtained for the riboflavin (B_2, G) content of neoplasms. In rat sarcomas, this vitamin was found in concentrations which equalled approximately one-seventh of that of normal liver, and the livers of tumor hosts were somewhat poorer in vitamin than the livers of normal rats.[218] The riboflavin concentration of Jensen rat sarcomas was given as 0.025—0.5 γ per gram of fresh tumor tissue, as compared with 1 γ per gram of normal rat muscle.[219] Similarly, hepatic tumors induced in rats by the ingestion of aminoazotoluene were found to exhibit a lower riboflavin content than normal hepatic tissue, but no deviation from the normal was observed regarding the riboflavin content of muscles of tumor-bearing animals.[220] Continued work led to analogous results: the riboflavin content of transplanted rat hepatomas was determined as being 25 γ per gram of dried tissue, whereas the corresponding value for normal rat liver was found to be 100 γ; low riboflavin values were also determined for Jensen rat sarcomas and for various mouse tumors.[220a] There can be no doubt that these findings have an important bearing on tumor metabolism, since riboflavin is the prosthetic group of an important oxidative system (Warburg's yellow respiratory enzyme).

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[213] W. Nakahara: J. Cancer Res. *13*: 363 (1929).

[214] M. T. Burrows & L. H. Jorstad: Am. J. Physiol. *77*: 24 (1926).

[215] T. Kubawara: Tr. Jap. Path. Soc. *18*: 612 (1929).

[216] E. Schneider & A. Burger: Klin. Wchnschr. *17*: 905 (1938).

[217] J. C. Abels, A. T. Gorham, L. Craver & C. P. Rhoads: J. Clin. Investigation *21*: 177 (1942).

[218] L. B. Brabec: Am. J. Cancer 25: 551 (1935).

The participation of vitamin B complex in spontaneous or induced tumor formation is a problem which has caused controversial claims. On the one hand, it was previously mentioned that several authors reported spontaneous tumor formation in the gastro-intestinal tract of animals fed a diet poor in vitamin A and rich in vitamin B,[173-175, 186] although it was stressed that other workers failed to confirm these findings and that they denied the malignant nature of the lesions produced in animals by means of the diet named. Nevertheless, reference must be made to studies according to which the alternating of a diet poor in vitamin A and rich in vitamin B, with a diet rich in vitamin A, produced adenocarcinomas in five per cent of rats treated in this manner.[182, 221, 222] Histologically, it was believed, a damage of the reticulo-endothelial system could be demonstrated in animals subjected to this regime,[223] and this phenomenon was thought to be responsible for a predisposition of the animals to neoplasia.[222] A connection may perhaps be assumed to exist between these observations and the report that injections of embryonic tissue admixed with arsenic acid failed to produce sarcomatous growth in fowls on a normal diet (cf. chapter I) but was successful in animals fed a diet rich in vitamin B.[224] In this connection it may be also mentioned that a metabolic alteration has been supposed to be responsible for the creation of a predisposition to neoplasia in consequence of the administration of the diet rich in vitamin B and poor in vitamin A. This concept was based on the observation that this dietary regime caused an increase of the carbon/nitrogen ratio in the urine, a phenomenon known as dysoxidative carbonuria, and this apparent disturbance of metabolic oxidation was believed to favor the development of tumors ([225], cf. Chapter VI). Yet it must be stressed that the stimulation of carcinogenesis by means of vitamin B has not been corroborated by other investigations. Thus pigeons kept on a diet free of vitamin B showed an increased tendency to the formation of spontaneous tumors [226] and, conversely, a diet rich in vitamin B failed to accelerate the tumor induction in mice by means of methylcholanthrene.[227] These divergent results may be

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[219] F. Vivanco: Sv. Vet. Akad. Ark. kem *12A*: No. 3 (1935); quot. H. v. Euler, M. Malmberg & G. Günther: Ztschr. f. Krebsforsch. *45*: 425 (1937).

[220] H. Kahler & E. F. Davis: Proc. Soc. Exper. Biol. & Med. *44*: 604 (1940).

[220a] W. V. B. Robertson & H. Kahler: J. Nat. Cancer Inst. *2*: 595 (1942).

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221. Rh. Erdmann, E. Haagen & K. Bernstein: Deutsche med. Wchnschr. *53*: 796 (1927).

222. Rh. Erdmann & E. Haagen: Med. Welt *2*: 141 (1928); Ztschr. f. Krebsforsch. *26*: 333 (1928).

223. E. Müller: Ztschr. f. Krebsforsch. *34*: 10 (1931).

224. V. Bisceglie: Riv. pat. sper. *8*: 364 (1932).

225. H. R. Kanitz & R. Simke: Ztschr. f. Krebsforsch. *45*: 39 (1937).

226. D. D. Chatterjee: J. Indian Med. Assoc. *5*: 5 (1935); abstr. Ztschr. f. Krebsforsch. *44*: Ref. 246 (1936).

227. T. Gordonoff & F. Ludwig: Ztschr. f. Krebsforsch. *47*: 421 (1938).

explained by various factors. Different processes of carcinogenesis and different animal species may be influenced in different ways; the partial components of the vitamin B complex may exert a different activity; and, finally, disturbances of the vitamin equilibrium may be responsible to a greater extent for the effects observed than the lack or the excess of a single vitamin in the diet.

Somewhat clearer aspects are exhibited by the results of studies dealing with the influence of vitamin B on tumor growth, as tested with transplanted tumors. Most workers observed a stimulation of the growth of inoculated tumors following the excessive administration of the vitamin B complex[9, 184, 193, 228, 229] or of vitamin B_1.[230] However, lack of the vitamin B complex in the diet did not appear markedly to inhibit the tumor growth,[42, 213] unless this vitamin deficiency severely impaired the general health of the animals.[42] Nevertheless, it has been reported that the growth of normal fibroblasts and of neoplastic cells was strongly impeded when the plasma of animals affected with beri-beri was used as the medium of the tissue cultures.[189] Some authors failed to notice a growth stimulation of transplanted tumors subsequent to the administration of vitamin B preparations,[185, 218] but these discrepancies have found a satisfactory elucidation by the following observation. The growth of mouse sarcoma 180 was influenced only to an insignificant extent by administration of B_1, B_2 or nicotinic acid, in spite of the effect of these substances on somatic growth. Yet the whole vitamin B complex markedly increased the tumor growth, for which effect vitamin B_6 and the filtrate factor (pantothenic acid) were tentatively held responsible.[231] The authors of the last named communication also reported that a diet

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228. B. Heymann & A. Gallinek: Zentralbl. f. Bakteriol. *106*: 73 (1928).
229. R. Nomura: Jap. J. Obst. & Gynec. *21*: 141 (1938).
230. W. Caspari: Ztschr. f. Krebsforsch. *38*: 361 (1932).
231. F. Bischoff & M. L. Long: Am. J. Cancer *37*: 54 (1939).

free of vitamin B arrested the growth of mouse sarcoma 180, but the original growth rate of the tumor was resumed after the return of the animals to a normal diet.[232] Regarding riboflavin (vitamin B_2 or G), injections of this substance were found to be without effect on rat sarcomas [218] and on the ascites carcinomas of mice,[233] but the addition of riboflavin to tissue cultures of Jensen rat sarcomas was reported to inhibit their growth and to cause a damage of the cells, which effects resulted in a failure of the treated cells to grow when re-inoculated into animals. The growth of embryonic cells did not appear to be affected by riboflavin.[234] The elimination of pantothenic acid from the diet strongly inhibited the growth of spontaneous mouse tumors and the resumption of a normal growth rate of the tumors was observed in the animals after the addition of the vitamin to the diet. A therapeutic utilization of this effect of pantothenic acid was not believed feasible, since the lack of this vitamin caused very severe damage to the health.[235]

The aforementioned opinion, that various carcinogenic processes may be affected in a different manner by the administration of certain vitamins, has found support in a number of studies in which the vitamin B complex or some of its components were found to inhibit the formation of chemically induced tumors. One of the first observations concerned the development of hepatic tumors in rats by means of butter yellow (cf. chapter II), a process which was markedly inhibited when the animals received a diet containing large quantities of yeast.[236–238] This effect was apparently dependent upon the high concentration of vitamin B complex in yeast. Exact re-examinations confirmed this phenomenon; while fifteen per cent of yeast in the food completely prevented the development of hepatic tumors, administration of smaller amounts of yeast resulted in a protective effect of lesser degree.[38] Attempts have been made to identify more closely the factors contained in the yeast which are responsible for the phenomenon under discussion, and a marked protection against the formation of hepatomas in rats fed butter yellow was found in animals receiving a diet rich in casein and riboflavin, although the tumor prevention caused by this diet was not as complete as that resulting from the feeding of yeast.[239] Experiments carried out by other workers on the influence of diet on tumor formation due to feeding of butter yellow led to very similar results,[84] yet the fact that the preventive effect of whole yeast was found superior to that of riboflavin, or other isolated vitamin B factors, strongly suggested that some unknown substances present in yeast are partly responsible for its activity. Vitamins of the B complex most likely were responsible for the prevention of the formation of hepatic tumors by means of butter yellow in rats which were fed rice bran oil,[240] or for an inhibition of the same carcinogenic process in rats fed millet.[241] Similarly, an inhibition of the hepatoma formation due to feeding of aminoazotoluene was observed in rats which received, instead of rice, a diet consisting of wheat or barley, a phenomenon most probably connected with the varying vitamin B content of these different cereals. It was tentatively suggested that such dietary factors may be responsible for the higher clinical frequency of hepatic tumors in the Far East.[242] Another series of investigations dealt with the influence of the vitamin B complex

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[232] F. Bischoff & M. L. Long: Cancer Research *1*: 217 (1941).
[233] H. Henkel: Ztschr. f. Krebsforsch. *51*: 199 (1941).
[234] C. Silva-Laflentz: Ztschr. f. Krebsforsch. *48*: 532 (1939).
[235] H. P. Morris & S. W. Lippincott: J. Nat. Cancer Inst. *2*: 47 (1941).
[236] R. Kinosita: J. jap. soc. diseases digestive organs *37*: 513 (1938).
[237] T. Ando: Gann *32*: 252 (1938).
[238] W. Nakahara, T. Fujiwara & K. Mori: Gann *33*: 57 (1939).

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239. C. J. Kensler, K. Sugiura, N. F. Young, C. R. Halter & C. P. Rhoads: Science *93*: 308 (1941).
240. D. Okada: Osake-Igaku-Zasshi *37*: 827 (1938).
241. S. Morigami & N. Kasiwabara: Gann *35*: 65 (1941).
242. K. Mori & W. Nakahara: Gann *34*: 48 (1940).

on tumor induction in mice painted with benzpyrene; feeding of yeast impeded this carcinogenic process,[243] yet the alimentary ingestion of thiamine, riboflavin, or nicotinic acid did not exert a marked effect, with the exception of a slightly retarded tumor development caused by thiamine.[244] In continued experiments, the animals were fed with high doses of thiamine and of pyrophosphates for three weeks before the administration of benzpyrene. This treatment was continued during the painting with benzpyrene and was found to impede the tumor development. A similar, but weaker, effect was noted from the feeding of nicotinic acid plus pyrophosphates, and the importance of phosphoric esters of these vitamins (prosthetic groups of coenzymes) for metabolic processes was stressed.[245] Although the results of these experiments were confirmed, it was not believed that the vitamins mentioned exert a true antiblastic effect, but rather that they aid in processes of detoxication of the carcinogens administered.[246] Such an assumption appears plausible, particularly as far as processes are concerned which involve an alteration of important metabolic enzymes in consequence of the administration of carcinogens; most likely, this also holds good for the prevention of the hepatic tumors previously enumerated. Somewhat different aspects are exhibited by investigations in which water-soluble yeast extracts of non-protein nature were employed (cf. chapter IX); injections of these preparations resulted in a complete cure of thirty per cent of spontaneous mouse cancers treated, but this effect was not believed to be due solely to the vitamin content of the extracts.[247] The intravenous injection of these extracts was, furthermore, found to impede the takes of inoculated mouse tumors, when the treatment started ten days prior to the transplantation. Under these experimental conditions, the addition of vitamins to the yeast extract increased the effectiveness of the treatment, as shown by the following figures indicating tumor growth prevented: yeast extract alone—twenty-one per cent, yeast extract plus riboflavin—sixty-two per cent, yeast extract plus thiamine—forty-eight per cent of "non-takes." It was, however, stressed that the administration of riboflavin, thiamine or pantothenic acid by themselves failed to influence the development of inoculated tumors.[248]

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[243] J. Maisin, Y. Pourbaix & P. Caeymaex: Compt. rend. Soc. de biol. *127*: 1477 (1938).

[244] J. Maisin, Y. Pourbaix & J. Camerman: Compt. rend. Soc. de biol. *130*: 1381 (1939).

[245] J. Maisin, Y. Pourbaix & J. Camerman: Compt. rend. Soc. de biol. *132*: 87 (1939).

[246] J. Maisin: Bull. Assoc. franç. p. l'étude du cancer *29*: 6 (1940).

A constituent of the vitamin B complex which has acquired great significance in recent times is biotin, one of the most active stimulants of growth known. Preliminary work seemed to indicate a high biotin concentration in malignant tumors,[249] but continued elaboration led to a revision of this assumption. The biotin distribution was examined in normal, fetal and corresponding neoplastic tissues by means of a bio-assay (effect on growth of micro-organisms). Shope papillomas and carcinomas of rabbits exhibited much higher biotin values than normal rabbit skin, but the values for fetal skin were found to exceed those obtained in the tumors. Two transplanted rabbit sarcomas also showed a higher biotin content than normal connective tissue of rabbits. On the other hand, reduced biotin values, as compared with the corresponding normal tissues, were observed in rat hepatomas induced by butter yellow, in transplanted mouse hepatomas, and in transplanted sarcomas of mice and rats. Human liver cancer contained less biotin than normal hepatic tissue, whereas the biotin values in a few cases of gastric cancer exceeded those of the normal gastric mucosa. In all these instances, the deviation from the normal observed in neoplastic tissues was paralleled by the behavior of the fetal tissues. It was thought possible that this fact may be connected with peculiarities of the carbohydrate metabolism common to tumor and embryo. The following circumstances were enumerated as speaking against a causal connection of biotin and the tumor process. Many tumors exhibit low biotin values; the same values were determined for benign and malignant tumors (Shope papilloma, and carcinoma, respectively); transplanted mouse hepatomas in the first stages contain the same amount of biotin as normal liver, whereas later stages of the tumor growth are accompanied by a considerable decrease of the biotin concentration. Finally, transplanted mouse sarcomas grew rapidly in animals which had been deprived of biotin by means of the administration of avidin, and tissue cultures of mouse sarcoma showed growth in the absence of biotin. The latter observations apparently indicate that tumor growth is independent of biotin.[250] On the other hand, an involvement of biotin in one special form of carcinogenesis has been demonstrated: rats were fed a diet with a strong protective quality against formation of butter yellow tumors.[cf. 33, 239] When avidin was added to this diet, no hepatomas developed in the animals receiving butter yellow. However, the addition of biotin to the protective diet resulted in the formation of hepatomas, and thus a cocarcinogenetic action of biotin was revealed.[251] It would, however, be premature to draw far-reaching conclusions from these interesting experiments; continued work must be awaited in which the effect of biotin on other forms of carcinogenesis is studied.

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[247] R. Lewisohn, C. Leuchtenberger, R. Leuchtenberger, D. Laszlo & K. Bloch: Cancer Research *1*: 799 (1941).

[248] R. Lewisohn, C. Leuchtenberger, R. Leuchtenberger, D. Laszlo & K. Bloch: Science *94*: 70 (1941).

[249] P. M. West & W. H. Woglom: Science *93*: 525 (1941).

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[250] P. M. West & W. H. Woglom: Cancer Research *2*: 324 (1942).

[251] V. du Vigneaud, J. M. Spangler, D. Burk, C. J. Kensler, K. Sugiura & C. P. Rhoads: Science *95*: 114 (1942).

Attempts to employ vitamins of the B complex for treatment of human neoplasia have not been recorded, with the one exception of a recent publication on the use of high doses of nicotinic acid (up to one gram daily) in human epidermoid carcinoma, especially of the oral cavity. Although the authors of this study declined to describe the results of this treatment as a "cancer cure," they reported on remarkable observations.[252] After prolonged peroral intake of nicotinic acid, sometimes used in combination with methylene blue, tumors of cauliflower type presented a marked cessation of growth, a demarcation from surrounding normal tissue and, sometimes, even a diminution in size; increased inflammatory infiltrations were also observed, whereas superficial ulcerative processes tended toward scabbing and epithelization. The favorable influence of nicotinic acid on the clinical condition of some of the tumors treated was shown, by means of biopsies, to be accompanied by far reaching changes in the histological structure of the cancerous growth, characterized particularly by an increase of keratohyalinization. The mechanism of these effects was tentatively assumed to be connected with the fact that nicotinic acid is a component of the coenzyme II (Warburg's coferment),[252] and thus may counteract disturbances of the tumor metabolism which are discussed in chapter VI. Reference may also be made to the chemical relationship of nicotinic acid to pyrimidine bases; these compounds, as well as nicotinic acid, have been found to stimulate the reticulo-endothelial function (cf. chapter VIII) and hence the beneficial action of the treatment may depend on an activation of this system. Actually, preliminary determinations of the reticulo-endothelial function in cancer patients treated with nicotinic acid seemed to bear out such an assumption.[253] At any rate, a continuation of these interesting experiments of a cancer treatment appears desirable, and further experiences may render possible a more definite evaluation.

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[252] R. Willheim & F. Bocobo: Acta med. Philipp. *2*: 445 (1941).

Vitamin C. This vitamin (cevitamic acid, ascorbic acid) represents a very powerful reducing agent and, while an extreme lack of vitamin C results in the well defined clinical manifestations of scurvy, lesser degrees of vitamin C deficiency apparently cause alterations of various enzymatic and metabolic processes, thus indicating a participation of this vitamin in a great many body functions.

Analytical determinations of the vitamin C content of miscellaneous human and animal neoplasms have been performed by means of the titrametric reduction method employing dichlorophenol indophenol, and in this manner comparatively high values for the vitamin were obtained.[254-258] Some authors believed they had evidence of a considerable discrepancy which becomes apparent when the titrametric or the biologic vitamin assays are applied to tumor tissue, inasmuch as the latter procedure indicated the presence of only small amounts of scurvy-preventing substances.[255, 259] These findings have not been confirmed in later studies in which—on the contrary—a very satisfactory agreement was found between the results of chemical and biological vitamin C determinations in Jensen rat sarcomas.[258, 260-262] At any rate, a

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253. R. Willheim: private communication.
254. E. Boyland: Biochem. J. *27*: 802 (1933).
255. L. J. Harris: Nature, London *132*: *27*; 605 (1933).
256. S. Edlbacher & A. Jung: Ztschr. f. physiol. Chem. *227*: 114 (1934).
257. G. E. Woodward: Biochem. J. *29*: 2405 (1935); J. Biol. Chem. *109*: *7* (1935).
258. R. R. Muslin, E. Silverblatt, C. G. King & G. E. Woodward: Am. J. Cancer *27*: *767* (1936).
259. B. Borghi & R. Deotto: Ztschr. f. Krebsforsch. *40*: 293 (1934).
260. A. E. Kellie & S. S. Zilva: Biochem. J. *30*: 1216 (1936).
261. C. Voegtlin, H. Kahler & J. M. Johnson: Am. J. Cancer *29*: 477 (1937).
262. A. F. Watson: Brit. J. Exper. Path. *17*: 122; 124 (1936).

high vitamin C concentration does not seem to be characteristic for all types of neoplasms, since only slightly increased values[263] or even rather low values were determined for various animal tumors.[262, 264, 265] A high vitamin C content found in hypernephromas was believed to be due to the fact that this tumor is derived from the adrenals, an organ with a high vitamin C concentration, and actually the values of ascorbic acid determined for other human neoplasms were much lower, ranging between 0.5 and 22.0 mg. per 100 gram of tissue.[266] Attention must be given to the observation that the ascorbic acid content of necrotic tumor parts was found much lower than that of intact tumor areas; in the latter, values were determined ten times greater than in the former,[256] and similar findings were reported by other workers.[265] For this reason, analytical determinations of vitamin C in malignant tumors should be performed preferably in non-necrotic material, and the neglect of this precaution may have been responsible for some of the divergent results mentioned. An estimation of the vitamin C content of the skin in vivo has been tried by means of intracutaneous injections of dichlorophenol indophenol and by measuring the time required for the disappearance of the blue color (reduction). In this manner a very short reduction time has been observed in skin cancer, but this occurs also in other pathologies such as lupus, herpes zoster, warts, etc. The accelerated disappearance of dichlorophenol indophenol was believed to indicate a high vitamin C content of skin cancers,[267] but it seems very doubtful whether this conclusion was justified, since the reduction of dichlorophenol indophenol was most probably caused not only by ascorbic acid but also by other reducing substances and by enzymatic factors. Accordingly, no real value can be assigned to this technique which, moreover, resulted in similar findings in malignant and nonmalignant pathologies.

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[263] R. Bierich & A. Rosenbohm: Ztschr. f. physiol. Chem. *231*: 47 (1935).

[264] A. Fujita, D. Iwatake & T. Miyata: Libro de Oro, Buenos Aires, p. 418 (1935).

[265] W. Stepp & H. Schroeder: Ztschr. f. d. ges. exper. Med. *98*: 611 (1936).

[266] M. Askanazy: Ztschr. f. Krebsforsch. *43*: 405 (1936).

[267] H. Rotter: Ztschr. f. Vitaminforsch. *8*: 24; 323 (1939).

Several investigations indicated a disturbance of the vitamin C distribution in the organism of tumor hosts. A diminution of ascorbic acid was found in the livers of tumor-bearing animals[256] and in many organs of rats inoculated with Walker carcinosarcoma 256.[268] The spleen of mice with transplanted tumors was reported to show an increase of vitamin C, whereas the adrenals of cancer patients were found to contain less ascorbic acid than the adrenals of persons free of tumors.[265] Furthermore, it appeared very probable that tumor hosts utilize greater amounts of vitamin C than healthy persons, since a diet free of vitamin C depleted the ascorbic acid content both of normal and of malignant tissues, but exhausted the reserves in tumor-bearing animals faster than in control animals.[262] A similar conclusion has been drawn from the observation that vitamin C injected parenterally is only incompletely excreted by cancer patients,[265] and analogous findings of a vitamin C retention in neoplastic diseases after the administration of high vitamin doses have been reported by several other workers.[269-272] Similarly, patients with metastatic cancer were believed to utilize greater amounts of vitamin C, since injections of the vitamin into these patients resulted in a lesser excretion of ascorbic acid

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268. B. Sure, R. M. Theis, R. T. Harrelson & L. Farber: Am. J. Cancer *36*: 252 (1939).
269. L. Bordoli: Tumori n.s. *11*: 532 (1937).
270. A. Jezler, H. Kapp & F. Ippen: Ztschr. f. klin. Med. *133*: 692 (1938).
271. S. Antes & C. Molo: Schweiz. med. Wchnschr. *69*: 619 (1939).
272. A. Vogt: Strahlentherapie *65*: 616 (1939).

than in control cases; the higher vitamin requirement of actively growing tissues—i.e., of the neoplasms—was thought to be responsible for this phenomenon.[272a] Other workers regarded the vitamin C deficiency of tumor patients as the expression of cachexia;[172, 270] however, it cannot at present be decided whether the retention of vitamin C, observed in cancerous patients, depends merely on general nutritional deficiencies or on more specific influences of the tumor process. The first assumption seems to be supported by a report, according to which the urinary excretion of vitamin C in cancer patients showed variations corresponding to those in normal persons, but the excretion markedly increased following blood transfusions.[269] Conflicting statements have been issued as to the effect of vitamin C injections on the vitamin content of tumors. On the one hand, guinea pig sarcomas were reported to exhibit a high selectivity for retaining the injected vitamin[274] but, on the other hand, no accumulation of vitamin C was found in mouse tumors after injection of the vitamin.[265] Further investigations of the problem are desirable since the divergent findings may have been due to differences in the tumor types tested or in the dietary regimes of the animals. Summarizing, it may be stated that a varying vitamin C concentration of malignant tumors and a higher vitamin content of actively growing tumor tissue was made very probable; alterations in the distribution and metabolism of vitamin C in tumor hosts have been frequently observed, but a clearer insight into the significance of these disturbances must be furnished by continued work.

A controversial problem is that of the influence of vitamin C on the neoplastic process. Some investigators reported that a very considerable stimulation of the growth of transplanted mouse tumors resulted from peroral or parenteral vitamin C administration,[275, 276] or they observed an acceleration of the malignant transformation of tar warts in mice receiving large amounts of vitamin C.[277] Likewise, in tissue cultures of Crocker mouse sarcoma 180 the addition of vitamin C promoted the mitotic activity and the cell migration and inhibited the cell degeneration,[278] all of which findings were also obtained in cultures of chick monocytes.[279] Yet a great number of authors observed only a slight stimulation of tumor growth from vitamin C administration [185] or failed to notice any effect of this measure on transplanted animal tumors.[256, 257, 263, 265, 280, 281] Similarly, the appearance of tumors induced in rats by means of dibenzanthracene was not found to be influenced by the administration of vitamin C.[282] It may be possible to explain these divergent findings on the basis of the assumption that the animals used in the first named studies [275-277] had suffered from a latent C-avitaminosis, and that the vitamin addition created conditions more favorable for tumor growth, whereas optimal conditions were already present, at the start, in those experiments in which the vitamin failed to stimulate tumor growth. However, it remains obscure which factors were responsible for the outcome of experiments in which vitamin C injections were reported to inhibit the growth of Kato rabbit sarcomas and the formation of metastases of this tumor,[283] or similarly to influence

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[272a] A. H. Minor & M. A. Ramirez: Cancer Research *2*: 509 (1942).
[273] W. G. Dencker: Strahlentherapie *67*: 143 (1940).
[274] E. Boyland: Biochem. J. *30*: 1221 (1936).

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[275] E. Fodor & St. Kunos: Ztschr. f. Krebsforsch. *40*: 567 (1934).

[276] F. Arloing, A. Morel & A. Josserand: Compt. rend. Soc. de biol. *120*: 205 (1935).

[277] D. L. Woodhouse: Biochem. J. *28*: 1974 (1934).

[278] J. P. M. Vogelaar & E. Erlichmann: Am. J. Cancer *31*: 283 (1937).

[279] L. E. Baker: Compt. rend. Soc. de biol. *120*: 932; 1160 (1935); *121*: 427 (1936).

[280] O. Hörner: Ztschr. f. d. ges. exper. Med. *99*: 570 (1936).

[281] G. Lancellotti: Biochim. e terap. sper. *25*: 540 (1938).

[282] J. A. Pollia: Radiology *25*: 338 (1935).

[283] R. Nomura: Jap. J. Obst. & Gynec. *21*: 144 (1938).

Brown-Pearce rabbit carcinomas.[284] In view of these conflicting statements it does not appear very encouraging to attempt a definition of the mechanisms by means of which vitamin C may influence the neoplastic process. In this connection it may be mentioned that ascorbic acid injected into normal guinea pigs was found to increase the glycogen content of the liver by sixty per cent,[285] and it may be surmised that such an accumulation of carbohydrates in the body represents a factor favorable for tumor growth. Furthermore, it is conceivable that ascorbic acid, by virtue of its affinity for oxygen, may be involved in the tumor metabolism to which a predominantly anaerobic character has frequently been ascribed (cf. chapter VI), but these considerations are admittedly speculative because they are not as yet supported by sufficient experimental evidence.

Regarding the therapeutical use of vitamin C, a group of authors proposed a cancer treatment which would employ various metal salts of dehydrascorbic acid, and they claimed they had obtained promising results from this therapy in animal and clinical experiments.[286-288] It seems necessary, however, to accept these statements with great reserve, on account of the very uncritical presentation of the alleged successes, and because of the lack of corroborating experiences of other workers with this treatment. Favorable effects on the general condition of cancer patients have been reported to follow the administration of vitamin C, but this treatment did

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284. G. Ryo: J. Chosen med. Assoc. *29*: 287 (1939).

285. L. Hirsch: Biochem. Ztschr. *287*: 126 (1936).

286. F. Arloing, A. Morel & A. Josserand: Compt. rend. Soc. de biol. *118*: 551; 1435 (1935); *120*: 201 (1935); Compt. rend. Acad. d. sc. *202*: 598 (1936).

287. L. Bérard, F. Arloing, A. Morel & A. Josserand: Lyon chir. *33*: 315; 593 (1936); abstr. Am. J. Cancer *31*: 513 (1937).

288. F. Arloing, A. Morel, A. Josserand & R. Chevallier: Lyon méd. *157*: 333 (1936); abstr. Am. J. Cancer *31*: 521 (1937).

not influence the tumor process proper [278, 289] and, obviously, is merely directed against a C-avitaminosis which may accompany neoplasia.

Vitamin D. There is a scarcity of analytical data pertaining to this vitamin in neoplasia. To the writers' knowledge, but one group of investigators have carried out such work and have found vitamin D present in transplanted rat and mouse tumors; in addition, they disclosed that the precursor of vitamin D, viz., ergosterol, is contained in these tumors in quantities exceeding those determined in the livers of rats and mice.[290]

Most of the experimental attempts to influence tumor growth by the administration of vitamin D were undertaken by virtue of the close connection of this vitamin with the mineral metabolism, that is, these studies were motivated by the expectation that the accumulation of calcium in tissues, which is brought about by vitamin D, may prove a factor counteracting the neoplastic growth. However, the results of several studies have failed to indicate that vitamin D, despite its leading to a calcium increase in tumor tissue, influenced the tumor process to any appreciable degree; [291-294] at best a slight diminution of the virulence of tumors was observed, following repeated transmissions to animals treated with the vitamin. Likewise, more recent experiments proved the administration of excessive amounts of vitamin D to be with-

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289. O. Kudlac & O. Storch: Wien. med. Wchnschr. *88*: 363 (1938).
(1931); abstr. Cancer Rev. *6*: 427 (1931); Gann *25*: 71 (1931).
290. M. Sumi & W. Nakahara: Sc. Pap. Inst. Phys. Chem. Res. *15*: 69
291. A. Goerner: J. Cancer Res. *14*: 545 (1930).
292. M. Sumi: Gann *24*: 239 (1930).
293. F. M. Brikker & Y. A. Lazaris: Vopr. Onkol. *4*: 174 (1931); abstr. Ztschr. f. Krebsforsch. *35*: Ref. 93 (1932); Ztschr. f. Krebsforsch. *37*: 423 (1932).
294. W. Caspari & F. Ottensooser: Ztschr. f. Krebsforsch. *37*: 423 (1932).

out effect on the growth of transplanted rat sarcomas,[295] and the presence or absence of vitamin D did not affect the growth of neoplastic tissue cultures.[189] These negative findings are not surprising, inasmuch as the tumor-inhibiting effect of calcium, as shown in chapter I, is principally confined to experiments in which calcium was used prophylactically. As a matter of fact, similar relations appear to hold good for the effect of vitamin D. According to one investigator, the administration of this vitamin to mice prior to the inoculation of tumors, or prior to painting with tar, markedly impeded the growth of the tumor transplant or the formation of tar cancer; existing tumors were not influenced by the vitamin.[296] In this connection it may be mentioned that a diet which produces rickets was found to stimulate the growth of transplanted tumors, but this effect could not be completely reversed by addition of adequate quantities of vitamin D to the diet.[297] The administration of large doses of vitamin D, continued for a longer period of time, was even seen to promote the growth of inoculated tumors and particular attention may be given to the disclosure that a tumor inhibition, which was observed in earlier experiments with vitamin D, in reality was due to the action of sesame oil, which was employed as the solvent for the vitamin.[294] This circumstance is liable to cause some doubt as to whether effects on the tumor process which have been observed from the administration of cod liver oil were caused solely by the vitamin D content of this substrate, or whether, instead, other constituents of the oil were not partly or mainly responsible for the outcome of such experiments. The latter assumption was held to be very probable by an author who observed a growth inhibition of Walker rat carcinosarcoma in animals kept on a cod liver oil diet, for

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295. Y. Hüzita: Jap. J. Obst. & Gynec. *21*: 382 (1938).
296. M. Barbiroli: Tumori n.s. *8*: 101 (1934).
297. W. Caspari & F. Ottensooser: Ztschr. f. Krebsforsch. *32*: 74 (1930).

he believed this effect to be due less to the vitamins of the oil than to the specific configuration of the fatty acids present in the oil.[75, 298] Earlier it was shown that feeding of cod liver oil to mice of an inbred strain with a spontaneous cancer incidence of fifty per cent depressed the tumor frequency to approximately thirteen per cent.[299] Other experiments in which cod liver oil was given to mice of strains with a low tumor susceptibility failed to affect the tumor frequency in these animals, but this negative result need not necessarily be regarded as being in conflict with the aforementioned observation, since it may be assumed that in the latter instances the high natural resistance of the animals could not be increased by the dietary factor.[2] Addition of cod liver oil to the food was reported also to inhibit the hepatoma formation in rats receiving o-aminoazotoluene.[299a] But it may once again be stressed that no direct evidence is available to prove that the effect of cod liver oil in the studies enumerated [298, 299] is to be explained by its vitamin D content, and the same is valid for some clinical attempts to use cod liver oil in the treatment of cancer. The external application of cod liver oil, enriched with vitamin D, has been proposed for the treatment of ulcerated skin cancer, and this procedure was reported to bring about granulations and epithelization of the tumors but did not markedly affect the tumor growth proper.[300] A greater effectiveness has been ascribed to cod liver oil irradiated with ultra-violet rays. Direct contact of this substrate with inoperable cancers of the uterine cervix was claimed to have caused a definite improvement and a prolongation of the life of the patients.[301] The small number

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298. F. L. Haven: Proc. Soc. Exper. Biol. & Med. *32*: 734 (1935).
299. E. Harde & N. Kobozieff: Compt. rend. Soc. de biol. *116*: 848 (1934).
299a. T. Ando: Gann *35*: 201 (1941).
300. R. Oppolzer: Wien. klin. Wchnschr. *47*: 30 (1934).
301. F. Orthner: Wien. klin. Wchnschr. *48*: 567 (1938).

of experiences recorded with this treatment, however, does not justify the acceptance of the theory of the author of this publication, who regarded a vitamin deficiency as the basic quality of malignant cells. Furthermore, it may be mentioned that some time ago an opposite type of cancer treatment was tried, that is, an elimination of the fat-soluble vitamins, to as great an extent as possible, from the diet of cancer patients; sixty-four patients with malignant tumors subjected to this dietary regime in some instances showed subjective improvement and gain of weight, but no appreciable effect on the tumor growth was observed.[302]

Finally, reference is made to studies of more recent date, which were undertaken on the basis of the chemical relationship between vitamin D and the polycyclic carcinogenic hydrocarbons, a relation which is expressed in the phenanthrene structure common to these compounds (cf. chapter II). These attempts to demonstrate a carcinogenic potency of vitamin-D-like substances met with success inasmuch as the introduction of irradiated ergosterol into the gallbladder of guinea pigs led to the formation of adenoid proliferations. Yet it must be stressed that non-irradiated ergosterol was ineffective, and that the malignant nature of the tumors mentioned has not been proved.[303] Painting of the skin of mice with irradiated ergosterol caused the formation of mammary adenomas in six out of fifteen animals after thirteen months, and subcutaneous injection of this compound induced lymphosarcomas in four out of thirteen animals.[304] However, also in these instances, the carcinogenic potency of ergosterol cannot be considered as being definitely established, in view of the scant number of experiments.

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302. S. Wyard: Lancet *1*: 840 (1922).

303. H. O. Schmidt: Klin. Wchnschr. *16*: 465 (1937); Ztschr. f. Krebsforsch. *45*: 335 (1937).

304. B. Polettini: Tumori n.s. *12*: 101 (1938).

Accordingly, a very doubtful theoretical foundation formed the basis for a study in which an "immunization" of rabbits was attempted by means of injections of vitamin D (supplemented by a hapten) prior to the inoculation of Brown-Pearce carcinoma. Moreover, the results of these experiments—lack of growth of the tumor transplants in thirty per cent of the animals treated[305]—cannot be regarded as very convincing, in view of the small number of animals tested and in view of the variability of the success of the transplantation in normal rabbits.

Vitamin E. Analytical determinations of this vitamin by means of chemical methods have only recently been rendered possible, and only a few studies of this kind have dealt with malignant tumors. Whereas it was first reported that comparatively high concentrations of vitamin E (α–tocopherol) were found in transplanted animal tumors (5 mg. per 100 grams of Jensen rat sarcoma, 4.4 mg. per 100 gram of Brown-Pearce rabbit carcinoma),[306] the same investigators later revised these data; by using improved methods they determined vitamin E values of 0.9 mg. per 100 gram in these tumors, i.e., concentrations corresponding to those found in normal organs rich in vitamin E.[307]

Regarding experimental work with vitamin E, several workers reported failure to influence the growth of transplanted tumors by means of the administration of this vitamin.[308–310] Likewise, experiments with rats in which a vitamin E deficiency was produced did not show any influence of this dietary disturbance on the growth of inoculated Walker carcinoma. Even continued re-inoculations (twenty-two trans-

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305. J. Štefl: Klin. Wchnschr. *16*: 501 (1937).
306. B. v. Euler & H. v. Euler: Ztschr. f. physiol. Chem. *264*: 141 (1940).
307. B. v. Euler & H. v. Euler: Ztschr. f. physiol. Chem. *265*: 147 (1940).
308. P. Engel: Ztschr. f. Krebsforsch. *39*: 148 (1933).
309. F. Marchesi: Riv. pat. sper. *11*: 396 (1933).
310. V. Zagami: Riv. pat. sper. *11*: 381 (1933).

missions performed during two and a half years) into vitamin E-deficient animals failed to cause any change of the biological or histological qualities of the tumors.[311] Only one author reported different results; that is, he noted a stimulation of transplanted tumors in female mice kept on a diet free of vitamin E, whereas the tumor growth was impeded in females receiving large vitamin doses. Male animals, however, reacted in the opposite manner: lack of vitamin E impeded, and administration stimulated, the tumor growth.[312] Although such an effect of vitamin E (antisterility vitamin), determined by the sex of the animal, is conceivable on account of its interrelationship with endocrines, the evidence presented in the aforementioned study does not appear strong enough to offset the negative conclusions which have been drawn from the other investigations on the influence of vitamin E on tumor growth, and for the time being this dietary factor cannot be regarded as being directly connected with neoplastic growth.

A similar stand may be taken regarding the effect of vitamin E on chemical tumor induction, because a report that a diet rich in vitamin E inhibited the formation of tar cancer in mice[313] cannot be accepted without reserve, since the diet employed in these experiments was not only rich in vitamin E but also changed in other respects (rich in vitamin B complex). A conflicting statement has been made according to which vitamin E promoted the development of tar cancer in mice; male and female animals were found to respond in the same manner, but the vitamin appeared to increase the resistance of the females against the toxic action of tar, whereas it lowered this resistance in males.[314] However, the majority of authors failed to notice any influence of vitamin E on the formation of tumors induced in mice by tar,[315] or induced in rats and mice by benzpyrene.[316, 317] Likewise neither the absence nor the administration of vitamin E affected the formation of methylcholanthrene tumors in mice,[318] nor did feeding to rats of vitamin E in the form of wheat germ oil influence the development of hepatic tumors induced by butter yellow.[319] Hence it appears very doubtful whether alimentary factors found to influence carcinogenesis can be considered as being connected with the vitamin E content of the diet.[315]

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[311] W. L. Bryan & K. E. Mason: Proc. Soc. Exper. Biol. & Med. *43*: 375 (1940).

[312] R. Severi: Pathologica *26*: 416 (1934).

[313] J. R. Davidson: Canad. M. A. J. *31*: 486 (1934); *32*: 364 (1935).

[314] R. Severi: Pathologica *27*: 524 (1935).

Because of experiments in which wheat germ oil was reported to be capable of producing tumors (cf. chapter II), the possibility of a carcinogenicity of vitamin E had to be considered, but studies using injections of, or painting with, vitamin E solutions led to completely negative results in mice and rats.[311, 316] On the other hand, evidence has been presented which was interpreted as indicating a tumor-producing property of a diet free of vitamin E. For it was observed that chicks fed a diet, the vitamin E content of which had been destroyed by ferric chloride, developed lymphoblastomas of the intestinal tract.[320] In continued work, formation of reticulum cell sarcomas was observed in chicks fed the diet free of vitamin E, but it was stressed that this occurred only when the animals at the same time received vitamins A and D in the form of cod liver oil or sardine oil.[321] This circumstance makes it probable to some degree that the tumor development was not due solely to the absence of vitamin E,

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315. A. T. Cameron & S. Meltzer: Am. J. Cancer *30*: 55 (1937).
316. V. Demole: Ztschr. f. Vitaminforsch. *8*: 341 (1939).
317. A. Haddow & H. Russell: Am. J. Cancer *29*: 367 (1937).
318. C. Carruthers: Am. J. Cancer *35*: 546 (1939).
319. K. Sugiura: Proc. Soc. Exper. Biol. & Med. *47*: 17 (1941).
320. F. B. Adamstone: Am. J. Cancer *28*: 540 (1936).
321. F. B. Adamstone: Arch. Path. *31*: 717 (1941).

but was also due to a disturbance of the vitamin equilibrium, an assumption which has also been held to be probable in connection with other intestinal lesions occurring in animals kept on a "vitamin-unbalanced" diet, as previously discussed. On the other hand the author of the aforementioned publications advanced a theory, according to which vitamin E is required for the normal metabolism of anthracene compounds occurring in the body (sterols, vitamin D, sex hormones), and according to which the absence of vitamin E may lead to the formation of carcinogenic substances from the compounds named.[322] This interesting conception, however, has still to be definitely established by more extensive experimental work.

3. *Dietary Treatment of Human Cancer.*

This section should present the practical conclusions which can be drawn from the data discussed in the preceding survey dealing with connections between nutritional influences and development and growth of malignant tumors; or, in other words, a rational dietary management of cancer patients should be outlined, a goal the attainment of which has been the obvious motivation of the work previously enumerated. Yet, as is generally known, this aim has been far from attained, a fact which may be explained by several circumstances. Above all, it must be considered problematic whether a general transfer of results obtained with dietary measures in animal experiments to human pathology is permissible, in view of the difference in the physiology of nutrition apparent in different species. This latter fact may partly account for divergent effects of the same nutritional agent in different animal species, inasmuch as it is conceivable that herbivorous, carnivorous or omnivorous animals may be distinguished by their response to the lack or the excess of some constituent in their diet. Furthermore, a clear distinction must be made between factors which influence the development of tumors, and those which affect tumor growth. In this connection, definite indications have been furnished that various carcinogenic processes are not uniformly influenced by nutritional factors, probably because the metabolic alterations caused by various carcinogenic stimuli follow different directions and hence they may be enhanced in one instance and impeded in another by the same dietary constituent. As far as human neoplasia is concerned, only little evidence, if any, is available to support the assumption that specific dietary regimes can be made responsible for the occurrence of this disease. Extensive statistical analyses of the relationship between nutrition and cancer[1] have not been able to change this outlook significantly, and one can but agree with the warning which has been voiced against drawing of unwarranted conclusions as to a connection between diet and formation of malignant tumors.[323] Unfortunately, the aspects exhibited by the influence of nutrition on tumor growth cannot be regarded as much more hopeful. In this regard only one fact appears to have been definitely established, namely, the promotion of tumor growth as a consequence of caloric overfeeding, and the opposite effect as a consequence of caloric restriction. The practical usefulness of the later phenomenon, however, is curtailed by the debilitating effect of undernourishment on the general health of tumor hosts.

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[322] F. B. Adamstone: Arch. Path. *31*: 722 (1941).

Despite the preceding remarks, dietary regimes for the treatment of cancer have been proposed by several authors who based their recommendations partly on the results of experimental work but, also, to a great extent, on their special theories of cancer origin, theories which have been discussed

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323. B. Sure: Hygeia *15*: 792 (1937).

and evaluated in other sections in this book. The discrepancies between the diets prescribed are numerous and, in general, it may be stated that most of the proposals entail severe restrictions of the intake of vital foodstuffs. By faithfully obeying all the rules laid down in this regard, a patient subjected to such dietary measures might be almost threatened with death by starvation and, at best, the dietary measures do not appear to be tolerated for any extended period of time. Furthermore, a precise assessment of the value of the dietary treatment is rendered practically impossible by the lack of publications in which their effect on a greater number of tumor patients has been critically analyzed. Instead of such a scientific procedure, general terms, such as subjective improvement, lessening of pains and other complicating symptoms, or prolongation of life, have mostly been used to describe the usefulness of dietary treatments.

In voicing this criticism it is not intended to deny that some of the recommendations for the nutrition of cancer patients may be helpful, but the need of continued work for improving their effectiveness is emphasized. The following enumeration refers to the most widely known dietary prescriptions in the treatment of cancer:

1) Freund and Kaminer[65] derived their cancer dietetics from their concept of the formation of cancer-promoting substances from palmitin and stearin in the intestinal tract, brought about by the action of pathological micro-organisms (cf. chapter X). Accordingly, they advocated the elimination of all animal fat from the diet and its replacement by vegetable oil (olive oil). Furthermore, the diet involves a restriction of carbohydrates in carcinomatous patients, or of peptones in sarcomatous patients. Frequent colonic irrigations and the peroral administration of disinfectant drugs (menthol, salol, bismuth subsalicylate) were believed to be effective means of normalizing the intestinal bacterial flora.

The authors themselves reported on favorable results achieved with this treatment in inoperable cancer patients,[324, 325] and similar experiences were announced by some other investigators.[326-328]

2) Caspari and Ottensooser[329] summarized their experimental results in the following proposals for dietary cancer treatment: liberal amounts of protein, restriction of carbohydrates, moderate amounts of vitamin A in the form of cod liver oil, minimal administration of vitamin B complex and vitamin C (exclusion of fresh fruit and vegetables). In particular, Caspari[2] regarded as a most important aim a stimulation of the reticulo-endothelial system, which he believed could be accomplished by changing the form of the nutrition every six months. Experimental support for the influence of different forms of nutrition on the reticulo-endothelial function has been derived from the results mentioned above,[222, 223] as well as from a study which showed that repeated changes in the form of nutrition caused an increased resistance of mice against tumor inoculation and, at the same time, demonstrated, by means of histological examination, an activation of the reticulo-endothelial system.[330]

3) De Raadt,[97] as mentioned above, ascribed to alkalosis the principal stimulating influence on the development and growth of tumors. Accordingly, the dietary measures he

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[324] E. Freund: Wien. klin. Wchnschr. *48*: 863 (1935); *50*: 541 (1937); Med. Klinik *33*: 1148 (1937).

[325] E. Freund & G. Kaminer: Wien. med. Wchnschr. *88*: 33; 64 (1938).

[326] J. Kretz: Wien. klin. Wchnschr. *48*: 1077 (1935); Med. Klinik *33*: 1149 (1937); Die krebsfeindliche Diät, Linz 1937; Monatschr. f. Krebsbekämpf. *5*: 247 (1937).

[327] B. Benda & J. Kretz: Wien. klin. Wchnschr. *49*: 174 (1936).

[328] R. Bayer: München. med. Wchnschr. *83*: 467 (1936); Wien. klin. Wchnschr. *51*: 506 (1938).

[329] W. Caspari & F. Ottensooser: Ztschr. f. Krebsforsch. *30*: 1 (1930).

[330] G. F. de Gaetani: Ztschr. f. Krebsforsch. *42*: 373 (1935); Bol. soc. ital. biol. sper. *10*: 908 (1935).

proposed aimed at the prevention of this shift of the pH by producing an acidosis.

4) Fischer-Wasels[881] adhered to a similar idea and detailed his recommendations in the following manner: the food should be poor in sugar, water, and salt, free of vitamin B_1 as far as possible, and restricted in the content of sterols, which he suspected are converted into carcinogenic compounds in the body. The acidification of the system should be achieved by means of administering hydrochloric acid, ammonium chloride and calcium phosphates.

5) Hoffmann,[1] in a comprehensive monograph mentioned at the beginning of this chapter, surveyed a great number of dietary regimes proposed for prevention of neoplasia or for the treatment of malignant tumors. He arrived at the conclusion shared by most authors that excessive food intake represents a factor contributing to the development and propagation of cancer and that, accordingly, the institution of a well balanced diet may be expected to be a helpful measure in combating neoplasia.

There can be no doubt but that the dietary measures which have so far been recommended as a supporting treatment of neoplastic diseases have by no means reached a stage where a definite value can be attributed to them. Nevertheless a number of experimental and analytical data, furnished by recent work, may be hoped to lead to a more satisfactory elaboration of this important problem.

CHAPTER VI

METABOLISM

THE relationship between descriptive biochemistry and the study of metabolism corresponds approximately to that between anatomy and physiology. In the case of general tumor pathology, prevailing opinion recognizes that a purely anatomical definition of this pathology is not sufficient, but that a consideration of patho-physiology must also contribute to the solution of the problem of cancer; similarly, within the scope of chemical cancer research, the investigations of chemical changes, i.e., of the metabolism, have almost overshadowed the value of purely descriptive tumor chemistry. It must. however, be conceded that the problems encountered in this field are extremely difficult, for it is not sufficient merely to establish possible deviations of the metabolism from the normal in existing neoplasms. Investigations must aim, pre-eminently, at demonstrating the occurrence of such alterations at a time when a cell starts to become malignant, difficult as it may be to recognize this event by means of other methods. Since the induction of malignancy is closely connected with certain changes of the growth pattern, this trend of work necessarily bears on relations between growth and metabolism. Studies of tumor metabolism must therefore cope with the problem of clarifying the pathology of a process, the physiology of which is still to a great extent shrouded in darkness. Yet, the possibility cannot be ruled out in this instance also, that—to quote an old saying (Krehl)—"pathology might become the teacher of physiology."

Students of tumor metabolism may avail themselves of two approaches, inasmuch as investigations are performed either in vivo or in vitro. The latter approach has led to considerable success but more definite significance may be attached to observations of metabolic processes in the living body. Aside from difficulties of technical performance, the evaluation of metabolic data obtained by direct study of the tumor host is complicated by the fact that these results are necessarily influenced by secondary factors not directly connected with the neoplastic process, such as cachexia, infection, anemia, destruction of vital organs, and involvement of endocrine organs. Hence, it is not surprising that metabolic studies performed in vitro yielded much more uniform results than those carried out in vivo.

The following survey is arranged in accordance with the customary classification of metabolism, i.e., proteins, lipids, carbohydrates and minerals are discussed. It may be remarked that emphasis has been placed on the processes of intermediate metabolisms, whereas in this connection problems of digestion or excretion have only a slight importance. First, the data for the neoplasms proper and then the relations pertaining to the tumor host will be described in the single sections. It is hardly necessary to add that numerous questions will arise; questions which are connected with enzymology or with nutrition, and which have been gone into in more or less detail in the chapters concerned with these subjects.

1. *Protein Metabolism.*

Nitrogen balance. The gauging of the quantitative relations of the protein metabolism in vivo by measuring the total intake of nitrogenous substances and comparing these values with the total nitrogen quantities, determined in the excreta during the same period of observation, is an established procedure. An approximately equal intake and output of nitrogenous compounds indicates a nitrogen equilibrium, whereas a negative nitrogen balance—loss of nitrogenous body constituents—results from a nitrogen excretion exceeding the nitrogen intake, or a positive nitrogen balance occurs when larger quantities of nitrogenous compounds are ingested than excreted. Generally speaking, a positive nitrogen balance is associated with processes of growth, inasmuch as this requires the formation of protein, whereas large-scale tissue destruction may be accompanied by a negative nitrogen balance. These circumstances indicate that the nitrogen balance in neoplasia will be influenced by two factors of opposite effects, namely, neoplastic growth and tissue breakdown, and the prevalence of either one of these processes may shift the nitrogen equilibrium in one or the other directions. Investigators who determined the nitrogen balance in cancer patients as long ago as 1889 believed themselves justified in assuming that a negative nitrogen balance is associated with cancer pathology apart from cachexia and secondary disturbances, and they held a hypothetical "cancer toxin" responsible for the loss of protein occurring in tumor hosts.[1, 2] Later examinations failed to prove that a negative nitrogen balance is regularly present in malignancy;[3, 4] under special conditions, cancer patients were found to maintain a nitrogen equilibrium.[5, 6] In recent times the nitrogen balance of rab-

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1. F. Müller: Ztschr. f. klin. Med. *16*: 496 (1889).
2. G. Klemperer: Ztschr. f. klin. Med. *16*: 550 (1889).
3. W. von Moraczewski: Ztschr. f. klin. Med. *33*: 385 (1897).
4. C. Lewin: Ergebn. d. inn. Med. u. Kinderh. *2*: 168 (1908).
5. A. Braunstein: Ztschr. f. Krebsforsch. *1*: 199 (1904).
6. Grafe & E. Wallerstein: Berlin. klin. Wchnschr. *51*: 286 (1914).

bits has been determined before and after the inoculation of Brown-Pearce carcinomas, but no characteristic change was observed to result from the tumor growth.[7]

Partial functions of the protein metabolism have formed the subject of studies dealing with the chemical nature of the ultimate protein breakdown products excreted in the urine of tumor hosts. Special attention was given to the urea excretion, a relative diminution of which was reported by several authors[8-11] although opposing views have been expressed.[cf. 4] A marked decrease of the urea excretion was found in rabbits nine to ten days after inoculation of Brown-Pearce carcinoma; this diminution ran parellel to the progressing tumor growth, and did not depend on liver or kidney metastases.[7] The reduced excretion of urea led one to expect an increase of other nitrogenous compounds in the urine, perhaps of ammonia. While the ammonia excretion was found actually to be increased in cachectic patients,[12] no regular increase of this substance was observed in non-cachectic cancer patients,[5, 13] and a study of recent date indicated only a moderately increased concentration of ammonia in the urines of patients with gastric cancer.[14] As was stated in chapter III, the urinary excretion of ammonium salts is generally found to be increased in conditions of acidosis, inasmuch as the ammonium base is used by the kidneys for the neutralization of acids excreted. Since the total nitrogen excretion in the urine of tumor patients was for the most part found to be unchanged,[4, 5, 7] it may be assumed that the urines of cancer patients or of tumor-bearing animals contain other products of protein decomposition, which normally are present in smaller quantities. Actually, an excretion of proteoses has been frequently noted in cancer patients,[12, 15] and several authors attributed a particular importance to protein disintegration products of larger molecular size, termed "colloidal nitrogen" or "oxyproteic acids." An increased concentration of these substances has been reported for the urines of tumor patients, but as described in detail in chapter II, the regularity and the primary connection of these findings with tumor pathology appears rather doubtful. Nevertheless, the not too numerous data available may be tentatively interpreted as indicating a two-fold disturbance of the protein metabolism in neoplasia. On the one hand, there seems to be an impaired ability of the tumor host to accomplish a complete breakdown of the protein molecule to components of low molecular weight and, instead, more complex compounds (proteoses, "colloidal nitrogen,") are excreted. On the other hand, the final products of protein metabolism may appear in concentrations deviating from the normal, as indicated by a diminished excretion of urea and an augmented urinary concentration of ammonia. Yet it is to be stressed that these assumptions have been neither definitely established by experimental work of recent date, nor has their primary connection with the tumor process been proved.

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[7] Th. A. Sverdlova: Arch. biol. Nauk *54*: 11 (1937); abstr. Ztschr. f. Krebsforsch. *50*: Ref. 315 (1940).

[8] Vogel: Ztschr. f. rationelle Med. *4*: 362 (1854); quot. loc. cit. 1.

[9] W. Rommelaire: J. d. méd., Bruxelles *77*: 84 (1883); quot. loc. cit. 1.

[10] H. Grégoire: Thèse de Paris, 1883; quot. loc. cit. 5.

[11] Kirmisson (1885); quot. loc. cit. 5.

[12] K. von Noorden: Lehrbuch der Pathologie des Stoffwechsels, Berlin 1905.

[13] G. Setti: Riv. venet. d. sc. med. *16*: 31 (1889); abstr. Zentralbl. f. inn. Med. *20*: 1007 (1900).

[14] E. Fischbach: Monatschr. f. Krebsbekämpf. *6*: 44 (1938).

The decomposition of cyclic amino acids is reflected in the urinary excretion of indole and phenol. These compounds have frequently been found in augmented concentrations in the urines of tumor patients, and also in the urines of those

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[15] H. Ury & E. Lilienthal: Arch. f. Verdauungskrankh. *11*: 72 (1905).

with cancers located outside the intestinal tract.[4, 16] It may be surmised that in these instances the formation of indole, which normally takes place in the intestinal tract, may be due to protein disintegration in the neoplasm. As a matter of fact, indicanemia has frequently been observed in cancer patients, particularly when extensive necrotic and destructive processes have set in, in the tumors.[17] In addition, the increased excretion of indole and phenolic compounds may be an indication of an impaired liver function. In this connection reference is made to the observation that the excretion of hippuric acid after administration of benzoic acid, viz., a test found to be very sensitive for detecting hepatic damages, reached the lowest values in metastatic liver cancer;[18] this apparently indicates a reduced ability of the liver to combine the benzoic acid with glycine. This is in satisfactory agreement with a recent report, according to which the simultaneous performance of several tests furnished definite evidence for the occurrence of hepatic dysfunction in a high percentage of patients with gastro-intestinal cancer. The impaired liver function was not thought to be entirely due to the presence of metastases in this organ, and a normal liver function was frequently found in patients after removal of their tumors.[19] Hence it may be tentatively assumed that such hepatic dysfunctions may in part be responsible for the mentioned deviations of the protein metabolism in tumor hosts.

An interesting change of the nitrogen metabolism has been detected in rats resistant to tumor transplantation, namely, a single roentgen irradiation administered to these animals

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[16] L. Servantie & A. Baron: Compt. rend. Soc. de biol. *118*: 147 (1935).

[17] St. Litzner: Med. Klin. *33*: 150 (1937).

[18] K. G. Kohlstaedt & O. M. Helmer; Am. J. Digest. Dis. & Nutrit. *3*: 459 (1936).

[19] J. C. Abels, P. E. Rekers, G. E. Binkley, G. T. Pack & C. P. Rhoads: Ann. Int. Med. *16*: 221 (1942).

caused a marked drop of their blood urea concentration, whereas such an effect from radiation did not occur in animals susceptible to tumors.[20, 21] An analogous reaction was also observed in mice which, by removal of a previously transplanted "tail tumor," had been made resistant against inoculation of Crocker sarcoma 180. These animals, as well as normal mice, showed the same average value of 30 milligrams of urea per 100 c. c. of blood, but after roentgen applications the urea level in the "immunized" animals dropped to 20 milligrams per 100 c. c., while no change took place in the control animals.[22] These results have been interpreted as indicating the involvement of metabolic processes in the phenomenon of tumor immunity (cf. chapter VIII), but no explanation can as yet be offered for the mechanism by which the urea metabolism is affected by the resistance of an animal to tumor inoculation.

Protein metabolism of tumors in vitro. One of the first investigations in this field disclosed that tumor slices possess an energetic activity in producing ammonia in vitro. Since this process was promoted by the presence of oxygen and the absence of sugar, it has been regarded as a reaction which makes energy available under these conditions.[23] The effect of suppressing ammonia formation was exhibited only by those carbohydrates which could be glycolyzed by tumors, that is, particularly glucose and mannose, whereas other sugars exerted only a slight influence, or none at all.[24] It must be stressed that similar observations were made in experiments with normal organs [25] and particularly with embry-

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[20] E. C. Dodds, W. Lawson & J. C. Mottram: Biochem. J. *19*: 750 (1925).

[21] E. C. Dodds: Am. J. Cancer *15*: 2765 (1931).

[22] R. H. Oster & W. T. Salter: Am. J. Cancer *32*: 422 (1938).

[23] O. Warburg, K. Posener & E. Negelein: Biochem. Ztschr. *152*: 309 (1924).

[24] W. T. Salter & C. D. Robb: Am. J. Cancer *22*: 87 (1934).

[25] N. C. Laclau & B. Braier: Compt. rend. Soc. de biol. *106*: 378 (1931).

onic tissues.[26] Metabolic measurements performed with tissue cultures of Walker rat sarcoma 319 demonstrated an increase of the incoagulable nitrogen in the medium of growing cultures,[27] a finding which most probably is related to the aforementioned observation. It appears significant that the ammonia-saving effects of the carbohydrates mentioned took place only in growing tissue cultures, but this phenomenon was not observed with resting cells.[28] This is in satisfactory agreement with the results obtained in the following study. In normal adult mouse liver, embryonic, growing, and regenerating liver, and malignant mouse tumors, the ammonia-saving effect of sugars was found to be directly proportional to the mitotic activity of the tissue tested.[29] On the basis of these results, it was thought very probable that the source of the formation of ammonia in these tissues was represented by a deamination of nucleic acids or their derivatives present in the nuclear structures of the cells, and hence the phenomenon described appears to be linked to the nucleoprotein metabolism. Roentgen irradiation of tissues in vitro was seen to increase the formation of ammonia, and in the case of neoplastic tissues this effect was found to be independent of changes of the glycolytic activity.[30] This result may indicate that certain processes of deamination in malignant tumors are not linked with the carbohydrate metabolism, in contrast to others for which such a connection has been established.

Although the preceding data refer principally to phenomena of protein cleavage, the obtaining ot precise information as to processes of protein synthesis in neoplasia is without doubt of great importance. Several investigators have presented evidence for the association of neoplastic growth with an augmented synthesis of proteins, and one author believed this phenomenon to be due to an increased oxygen consumption which he observed in the first stages of tumor induction.[31] This concept is seemingly in contrast to disturbances of oxidation which have been demonstrated as occurring principally in the carbohydrate metabolism of tumors, but this discrepancy could perhaps be explained by the assumption of a faulty utilization of oxygen by the neoplasms. That is, it is conceivable that the oxidative alteration of tumor metabolism is not characterized by the quantitative factor of oxygen consumption, but rather by a qualitative alteration, inasmuch as oxygen is used for protein synthesis instead of for carbohydrate cleavage. From another point of view it was shown that the hydrogen ion concentration in malignant tumors, which may be shifted to the acid side in consequence of glycolysis (cf. chapter III), represents very favorable conditions for protein synthesis, and these relations are obviously of great importance for the rapid growth of tumors.[32] The authors of the last-named study performed extensive investigations on the conditions under which proteolytic enzymes of normal and of neoplastic tissues bring about protein cleavage or protein synthesis. This work has been discussed in detail in chapter IV. Direct experimental proof for the increased protein synthesis in tumors has been furnished by a recent study. Normal rat liver pulp and Jensen rat sarcoma were incubated for 5 to 28 hours at 37° C under oxygen aeration. Before and after this incubation, determinations of the protein content, of the incoagulable nitrogen, and of the amino nitrogen were performed. As indicated by an augmentation of precipitable protein and a diminution of the nonprotein-nitrogen, a protein synthesis took place under these experimental conditions but, significantly, this synthesis was found twice as high in the rat tumor as in the normal liver.[33] It would be very desirable to extend similar investigations to precancerous lesions induced by carcinogens, and in this manner additional information could be gained as to the protein metabolism in neoplasia.

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[26] F. Dickens & G. D. Greville: Biochem. J. *27*: 1123 (1933).

[27] C. L. Gemmil, G. O. Gey & R. Austrian: Bull. Johns Hopkins Hosp. *66*: 167 (1940).

[28] E. Watchorn & B. E. Holmes: Biochem. J. *21*: 1391 (1937).

[29] J. Litter, B. B. Marble & W. T. Salter: Am. J. Cancer *31*: 268 (1937).

[30] H. G. Crabtree: Biochem. J. *30*: 2140 (1936).

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[31] P. Rondoni: Acta Cancrol. *2*: 205 (1936).

[32] M. E. Maver, J. M. Johnson & C. Voegtlin: Nat. Inst. Health Bull. No. 164: 29 (1935).

2. *Lipid Metabolism.*

One of the oldest references to fat metabolism in neoplasia may be found in Freund's theory,[34] which regards processes of fat digestion as being a decisive factor of development and growth of tumors. Since detailed discussions of this conception will be found in chapter X, it need only be mentioned that the authors assumed that, in cancer patients or in individuals predisposed to neoplasia, an unsaturated dicarboxylic acid is formed from ingested animal fat by the intestinal bacteria. This substance was held to be responsible for the antilytic action of the serum of cancer patients in the carcinolytic test, whereas the carcinolytic activity of normal sera was believed to be due to a saturated dicarboxylic acid produced in the intestines of normal persons. Yet, as stated in chapter X, the validity of these claims of chemical and metabolic changes appears very doubtful in the light of continued work. Credit may be given to Freund, nevertheless, for having been the first to call attention, in connection with tumor pathology, to fat metabolism—a problem which in general has found only slight elaboration.

Some support for the assumption of a disturbed intermediate fat metabolism may be seen in the following report. Whereas, fat intake normally causes an increase of ketone bodies in the blood, particularly of β-hydroxybutyric acid, this effect was found to be absent in cancer patients; that is, the concentration of ketone bodies in the blood failed to increase following the ingestion of fats.[35] Although this interesting observation has not formed the subject of continued studies, and hence has not been confirmed on a broader basis, some additional evidence for an alteration of intermediate fat oxidation may be derived from analytical findings. For it has been stated that fatty acids contained in the blood of cancer patients possess a higher molecular weight than those present in normal blood;[36] this increased molecular weight may be interpreted as being a consequence of a disturbed chain-reduction of the fatty acids (β-oxidation). Furthermore, the observation of increased concentrations of unsaturated fatty acids in the blood of cancer patients,[37, 38] cf. chapters II and X may indicate an interruption of the fat oxidation after dehydrogenation. More concrete evidence for alterations of the fat metabolism in malignant tumors has been derived from studies in vitro. Slices of human neoplasm, of Ehrlich mouse carcinoma, and of Rous fowl sarcoma were found incapable of oxidizing, in a normal manner, monocarboxylic fatty acids (saturated or unsaturated); sometimes even a reduction of the oxygen consumption was observed after the addition of fatty acids.[39] In continued work these results were supplemented by the disclosure that the ω-oxidation of fatty acids in neoplastic tissue takes place in the same manner as in normal tissues, whereas the β-oxidation was

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[33] B. I. Schomin: Bull. Biol. et Méd. Exper. URSS 7: 330 (1939).

[34] E. Freund & G. Kaminer: Biochemische Grundlagen der Karzinomdisposition, Springer, Vienna 1927.

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35. Gaessler: Zentralbl. f. Gynäk. *58*: 258 (1934).
36. Y. Pourbaix & R. Buytaert: Compt. rend. Soc. de biol. *115*: 1408 (1934).
37. M. Ascoli: Klin. Wchnschr. *14*: 1593 (1935).
38. R. Indovina & S. Fiandaca: Acta Cancrol. *1*: 399 (1935).
39. E. Ciaranfi: Am. J. Cancer *32*: 561 (1938).

found absent.[40] A further elaboration of these significant results may be expected with interest.

Data pertaining to the phospholipid metabolism of neoplasms have been discussed in chapter II. It may be repeated here that, according to experimental findings, phospholipids principally supply a structural function in malignant tumors as they also do in muscle, whereas in liver they participate to a greater extent in metabolic functions.[41] Furthermore, the rate of the turnover of phospholipids has been determined for various animal tumors by means of radioactive ("labelled") phosphorus.[42, 43] A continued investigation of the phospholipid metabolism appears very desirable in view of the importance of these compounds for the chemical and physicochemical qualities of the cell membranes.

The sterol metabolism of tumor-bearing animals has only recently undergone a closer examination, but conflicting results have been obtained from the few experiments dealing with this subject. On the one hand, determinations of the cholesterol balance in normal mice and in animals with transplanted tumors were reported to have shown only an insignificantly increased sterol synthesis as taking place in the latter animals as compared with tumor-free mice. The excretion of sterols in the feces was unchanged in tumor-bearing mice, and the animals were found not to store cholesterol added to the diet to a greater extent than did normal animals.[44] On the contrary, young rats, bearing Walker carcinoma 256, when fed a synthetic low-sterol diet, consistently synthesized more sterol than did normal litter mates free of tumors and fed the same diet; that is, the sterol excretion was found equal in both animal groups, but since the bodies of tumor-bearing animals contained larger sterol concentrations than did normal rats, this accounted for a higher sterol synthesis by the tumor-bearing animals.[45] It cannot be decided whether these discrepancies are due to the use of different species of animals, or whether they are due to differences in the experimental arrangements. In any event, clarification of this problem requires additional experimental analysis, and it may prove illuminating to study the sterol metabolism in animals also during the process of chemical tumor induction.

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[40] E. Ciaranfi: Arch. d. sc. biol. *26*: 8 (1940).

[41] F. L. Haven: Proc. Am. Soc. Biol. Chem. 13th meet., p. 47 (1936); J. Biol. Chem. *118*: 111 (1937).

[42] H. B. Jones, I. L. Chaikoff & J. H. Lawrence: J. Biol. Chem. *133*: 319 (1940).

[43] F. L. Haven: J. Nat. Cancer Inst. *1*: 205 (1940).

[44] F. L. Breusch: Am. J. Cancer *36*: 609 (1939).

3. *Carbohydrate Metabolism.*

In this field of metabolism of neoplasia the most impressive results have been accomplished and the great amount of data recorded on the subject leaves one almost with the impression that a nearly complete knowledge of these metabolic processes has been established. The vast material available on the subject, even more than ten years ago, was summarized at that time in reviews [46, 47] which will prove of great value to readers interested in this problem.

Glucose tolerance test. A discussion of the carbohydrate metabolism of tumor hosts may be started by mentioning the phenomenon of alimentary hyperglycemia, since the sugar tolerance test derived from it has undergone an extensive elaboration in connection with diabetes mellitus, and hence precise data are available as to the theoretical background and the experimental details in physiology and pathology. As remarked in chapter II, several reports refer to a hyperglycemia of fasting tumor patients, and hence a disturbance of the carbohydrate metabolism in neoplasia has been sus-

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45. E. R. Norris & E. E. Troescher: Cancer Research *1*: 410 (1941).
46. F. Dickens: Cancer Rev. *6*: 57 (1931).
47. E. C. Dodds: Am. J. Cancer *15*: 2765 (1931).

pected. Actually, a great number of authors observed a deviation of the blood sugar curves obtained in tumor patients after peroral glucose administration. They found an increased upper level of the rise of the blood sugar, and a delayed drop of this value, thus resulting in a flattening out of the curves.[48–65] The interpretation of these findings, however, gave rise to conflicting opinions. One extreme is represented by the view that the change of the blood sugar curve is a very regular phenomenon in cancer patients. Not only a diagnostic usefulness was ascribed to this deviation from the normal, but it was even believed to indicate a predisposition to neoplasia, since similar results of the sugar tolerance test were claimed to be obtainable in persons clinically free of tumors, but whose families showed a hereditary tendency to neoplastic diseases.[66] These far-reaching implications met

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48. J. Friedenwald & G. H. Grove: Am. J. M. Sc. *163*: 33 (1922).
49. S. Cytronberg: Polsk. Arch. Med. 1924: No. 3.
50. A. Foerster & C. Foerster: Klin. Wchnschr. *4*: 1540 (1925).
51. G. Scherk: Klin. Wchnschr. *5*: 1459 (1926).
52. I. Gavrila & T. Sparchez: Compt. rend. Soc. de biol. *97*: 1019 (1927).
53. F. Silberstein, J. Freud & T. Révész: Biochem. Ztschr. *181*: 305 (1927).
54. A. Slosse & R. Reding: Bull. Acad. d. sc. belg. *7*: 405 (1927); Cancer, Bruxelles, *4*: 442 (1927).
55. F. Pellegrino: Riv. di pat. sper. *3*: 246 (1928).
56. H. J. John: Med. J. & Rec. *131*: 287 (1930).
57. M. d'Halluin: Bull. et Mém. Soc. de radiol. méd. de Françe *18*: 170; 249 (1930).
58. P. Beregoff: J. Cancer Res. *14*: 559 (1930).
59. F. Benso: Tumori n.s. *4*: 425 (1930).
60. S. Choldin: Vopr. Onkol. *3*: 237 (1930); abstr. Ztschr. f. Krebsforsch. *34*: Ref. 123 (1931).
61. B. Enocksson: abstr. Am. J. Cancer *15*: 365 (1931).
62. V. Romanin: Minerva med. *22*: 870 (1931).
63. J. H. Roe & H. M. Dyer: Am. J. Cancer *15*: 725 (1931).
64. D. Jackson & D. A. Todd: Texas State J. Med. *30*: 197 (1934).
65. D. G. Krizchin, L. M. Erusalimski & S. I Aisenberg: Med. Ž. všeukrain Nauk *4*: 1153 (1935): abstr. Ztschr. f. Krebsforsch. *44*: Ref. 68 (1936).
66. R. Reding: Le Terrain Cancereux and Cançérisable, Masson, Paris 1933.

with justified objections, and several studies clearly demonstrated that the changes of the blood sugar curve described are by no means found in all cancer patients tested.[67-69] Furthermore, the primary connection of the results of sugar tolerance tests with the tumor process must be regarded as very doubtful, since it is known that a depletion of the hepatic glycogen reserves results—in the absence of malignancy—in a delayed return of the blood glucose level to the initial value, after sugar intake. Hence nutritional disturbances may be partly responsible for the abnormal reaction of tumor patients in sugar tolerance tests, and this view is supported by the statement that the changes are observed in a most marked form in patients with gastro-intestinal cancers.[49] A direct hormone influence, viz., of insulin, may be assumed to be responsible for the observation of irregular blood sugar curves in patients with carcinomas of the pancreas head, or of the biliary ducts.[70] In patients with skin cancer, blood sugar curves have been determined by the simultaneous testing of blood withdrawn from the finger tips of the patients, and of blood obtained from the tumor area. In the latter instance, higher initial blood sugar values and a more delayed return of the blood sugar were noted than was the case in the tests employing blood from the fingertips,[71] but these observations may have been due to an impaired blood circulation in the tumor area. The inconstancy of the sugar tolerance test in neoplasia has been clearly demonstrated in a recent examination of 128 tumor patients. The results of the test were not found to exhibit a direct relation to malignant disease.

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67. H. Biermer: Deutsche med. Wchnschr. *50*: 597 (1924).
68. G. Galli & R. Pecco: Miberva med. *18*: 925 (1927).
69. F. H. L. Taylor & H. J. Jackson, Jr.: Am. J. Cancer *22*: 536 (1934).
70. E. Meulengracht: Hosp. tid. *2*: 1145 (1930); abstr. Ztschr. f. Krebsforsch. *34*: Ref. 59 (1931).
71. G. Paltrinieri: Atti 3. conv. naz. per la lotta contro il cancro, p. 727 (1934); abstr. Ztschr. f. Krebsforsch. *43*: Ref. 185 (1936).

Only when taken as a group was the blood stream of patients with highly anaplastic tumors unable to be rid of an excess of glucose as quickly as that of patients with less anaplastic tumors.[72] Likewise, animal experiments proved that the results of glucose tolerance tests are not primarily dependent on the existing tumor; rabbits inoculated with Brown-Pearce carcinomas showed a greater rise of the blood sugar curves, but this was also observed in animals with regressing tumors. Since, furthermore, a similar change occurred in animals after the production of radiation necrosis and after injection of peptone or of tissue pulp, it was concluded that this phenomenon is due to the resorption of products of tissue breakdown, i.e., that it represents only a secondary effect of neoplasia.[73] This may also explain the fact that roentgen irradiation has been found to increase the hyperglycemia of tumor patients,[74] probably because this treatment causes destruction of tissues. On the other hand, it was reported that blood sugar curves approaching the normal could be obtained in patients whose tumors had been cured by irradiation therapy.[66, 75] Concerning the mechanism responsible for the impairment of the sugar tolerance by resorption of tissue breakdown products, an involvement of the reticulo-endothelial function is suggested by the disclosure that abnormal blood sugar curves—similar to those found in tumor hosts—were also observed in animals after splenectomy or blockade of the reticulo-endothelial system.[76-78] At any rate, the available evidence does not indicate that the results of sugar tolerance tests in neoplasia regularly deviate from the normal, or

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72. G. L. Rohdenburg: Cancer Research *1*: 311 (1941).
73. J. Loiseleur & W. Nyka: Compt. rend. Soc. de biol. *124*: 701 (1937).
74. F. Gentil: Compt. rend. Soc. de biol. *88*: 1323 (1923).
75. A. Kostygova: Vestn. rentgenol. *9*: 122 (1931); abstr. Ztschr. f. Krebsforsch. *36*: Ref. 91 (1932).
76. T. Togawa: Biochem. Ztschr. *109*: 1 (1920).
77. A. Noma; quot. K. Fuziwara: Biochem. Ztschr. *256*: 384 (1932).
78. A. V. Marx: Klin. Wchnschr. *9*: 2058 (1930).

are primarily connected with the tumor process, but rather that they represent a secondary phenomenon in those cases where they are detected.

Regulation of carbohydrate metabolism. In addition to the disturbances discussed in the foregoing, i.e., the hyperglycemia and the raised blood sugar curves of cancer patients, a few other observations serve as a basis for the assumption of an abnormal regulation of the carbohydrate metabolism in neoplasia. As will be described in chapter VII, according to some clinical observations a decrease of the glycosuria of diabetic patients takes place after they have contracted malignant tumors. The interpretation of these findings, however, meets with difficulties, since an appreciable change of the blood sugar concentration was not found in these patients, nor did phlorizin tests indicate that the renal threshold for glucose excretion was raised in diabetics with neoplasms.[79] No conclusive results have been obtained which would prove that tumor hosts respond in a characteristic manner to the administration of insulin or of adrenaline, thus distinguishing them from normal persons (cf. chapter VII) and, accordingly, it is unlikely that endocrine disturbances are directly responsible for a faulty regulation of the carbohydrate metabolism in neoplasia. One investigator regarded the metabolic disturbances in tumor hosts as being due to "a pathologically changed excitability of the organs governing the sugar metabolism," [80] but if this were to imply that the central nervous or autonomous nervous stimuli are affected by the tumor process, no direct experimental evidence has as yet been furnished for such an assumption. In connection with the sugar tolerance of tumor patients, controversial statements have been recorded. On the one hand, an increased carbohydrate tolerance was reported for these patients,[81] whereas other authors observed an increased tendency to glycosuria in tumor patients after sugar intake.[64, 66] The latter phenomenon may be interpreted as a secondary effect, as suggested by experiments with tumor-bearing rats. These animals were found to exhibit a diminished galactose tolerance, but this disturbance was recognized as being due to a hepatic dysfunction, since other liver functions were also impaired, as indicated by bilirubinemia, urobilinemia and alterations of the water metabolism.[82] Accordingly, disturbances of the carbohydrate metabolism observed in tumor hosts may be connected with disorders of the liver; this recalls similar conclusions advanced for certain aspects of protein metabolism, and this may explain why these changes of the metabolism are not a constant feature in tumor patients.

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[79] C. Wetzler-Ligeti & M. Köstenblatt: Wien. klin Wchnschr. *49*: 1047 (1936).

[80] F. Silberstein: Schweiz. med. Wchnschr. *62*: 153 (1932).

Sugar consumption in vivo. A small number of investigators attacked this interesting problem by comparative glucose determinations in the blood of tumor arteries and tumor veins, or in the blood of vessels supplying the tumor area and in the blood of vessels far from the site of the tumor. The results obtained clearly indicated that malignant tumors consume large quantities of glucose, since higher sugar concentrations were found in the artery supplying a Rous sarcoma than in the vein draining this tumor, and lower values were observed in blood derived from the tumor-bearing wing of fowl than in the tumor-free one.[83, 84] Similar differences were detected by glucose estimations performed during the surgical removal of human neoplasms, such as uterine and breast cancers.[85] It is noteworthy that, running parallel to the sugar decrease caused by the tumor metabolism, an increase of the lactic acid concentration has been found in the blood leaving the tumor area. The significance of this phenomenon becomes clear in the light of studies dealing with the carbohydrate metabolism of neoplasms in vitro.

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[81] N. Medvedeva: Ž. Exper. Biol. & Med. *4*: 790 (1927); abstr. Ztschr. f. Krebsforsch. *25*: Ref. 61 (1927).

[82] J. Putnoky & S. Sümegi: Ztschr. f. Krebsforsch. *41*: 505 (1935).

[83] K. Tadenuma, S. Hotta & J. Homma: Biochem. Ztschr. *137*: 536 (1923).

[84] C. F. Cori & G. T. Cori: J. Biol. Chem. *65*: 397 (1925).

Warburg's work on tumor glycolysis. Great credit must be given to Warburg and his collaborators[86–91] for their studies on the carbohydrate metabolism of malignant tumors in vitro, studies which led to the discovery of characteristic metabolic properties inherent in neoplastic tissues. These results, outlined in the following, may be regarded as one of the most valuable accomplishments of biochemical cancer research, since they convey an illuminating and comprehensive picture of an important phase of tumor metabolism. The value of the work in question is heightened by the fact that it has led to the establishing of quantitative factors, capable of being precisely calculated, and the methods employed are exact and easy to reproduce. The significance of this experimental work is not essentially impaired by the fact that it necessarily exhibits a somewhat one-sided approach to tumor pathology, and that theoretical conclusions derived from it have afterwards been found to be incapable of serving as a basis for a solution of the cancer problem proper; that is, attempts to utilize the metabolic alterations demonstrated in neoplasms for solving the cancer etiology, or for creating a

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85. A. Pistuddi: Riv. ital. ginec. *9*: 484 (1929); abstr. Cancer Rev. *6*: 27 (1931).

86. O. Warburg: Biochem. Ztschr. *142*: 317 (1923); *160*: 307 (1925); Klin. Wchnschr. *4*: 12 (1925); Über den Stoffwechsel der Tumoren, Springer, Berlin 1926.

87. O. Warburg & S. Minami: Klin. Wchnschr. *2*: 776 (1923).

88. S. Minami: Biochem. Ztschr. *142*: 334 (1923).

89. E. Negelein: Biochem. Ztschr. *158*: 121 (1925).

90. Y. Okamoto: Biochem. Ztschr. *160*: 52 (1925).

91. O. Stahl & O. Warburg: Klin. Wchnschr. *5*: 1218 (1926).

cancer therapy, have, in general, not met with definite success.

The basic point from which Warburg started his work is the simultaneous quantitative determination of oxygen consumption (respiration) and of glycolysis in neoplastic tissues. For a better understanding of the following statements, a brief outline of the physiology of intermediate sugar metabolism seems appropriate. For details and more thorough analyses of the subject, the reader is referred to text books on physiological chemistry (cf. also [92]). The sugar cleavage may be divided into two clearly discernible phases: the first comprises the transformation of a hexose into 3-carbon-compounds (methylglyoxal, glyceric acid, glyceric aldehyde, pyruvic acid, lactic acid), the second entails a subsequent complete oxidation of certain amounts of the 3-carbon-compounds and, at the same time, a partial re-synthesis of these to the hexose. Definite quantitative laws govern the ratio between re-synthesis and oxidation. The separation of these two phases is given by the fact that the processes of the first phase take place in the absence of oxygen, i.e., during anaerobiosis, whereas oxygen is required for the processes of the second phase. The details of these mechanisms were first established by Neuberg for the fermentation of yeast, but were also proved to be valid for general biology by the work of Meyerhof, Lohmann and many other authors, inasmuch as a striking similarity was found between sugar cleavage in yeast and the same process in muscle and other organs. The first phase of the hexose cleavage, which generally leads to lactic acid as the end product, is designated as glycolysis, and the quantitative determination of this compound played a basic part in the studies to be listed and described.

Warburg's work is based on a comparison between the values of glycolysis of tissues obtained either in the absence of oxygen (anaerobic glycolysis), or in the presence of oxygen (aerobic glycolysis). Anticipating the final results of Warburg's studies, his principal discovery is to be seen in the observation that the suppression of glycolysis, which occurs quite generally in normal tissues in the presence of oxygen, fails to occur in neoplastic tissues, or is of a much smaller extent than in normal tissues. While details of the technical carrying out of these measurements may be found in special monographs,[85, 93] it may be briefly mentioned that the principle of the methods employed is based on the fact that manometry makes possible a direct estimation of the oxygen consumed and of the carbon dioxide formed and an indirect determination of the lactic acid produced. A number of standard values and ratios had to be introduced, by means of which Warburg defined the metabolic activity of the tissues tested. The first, Q_{O_2}, refers to the oxygen consumption (respiration); the second, $Q^{N_2}_{LA}$, represents the lactic acid production in the absence of oxygen, i.e., the anaerobic glycolysis; and the third, $Q^{O_2}_{LA}$, represents the lactic acid production in the presence of oxygen (aerobic glycolysis). All these values refer to products formed by one milligram of dried tissue as calculated for one hour, and are connected by the following ratios. The difference between anaerobic and aerobic glycolysis, expressed as percentage of the anaerobic glycolysis, is given in the formula

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[92] D. Burk: Cold Spring Harbor Symposia on quantitative Biology, 7: 420 (1939).

$$\frac{Q^{N_2}_{LA} - Q^{O_2}_{LA} \times 100}{Q^{N_2}_{LA}}$$

and can be taken as a measure of the ability of respiration to suppress glycolysis in the respective tissues tested. The ratio between oxygen consumption and the amount of lactic acid caused to disappear by the respiration is shown as

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93. M. Dixon: Manometric methods, University Press, Cambridge (England) 1934.

$\frac{Q_{LA}^{N_2} - Q_{LA}^{O_2}}{Q_{O_2}}$, and indicates the size of the effect of one oxygen molecule used to suppress glycolysis. This ratio is designated as the Meyerhof quotient. Finally, Warburg established the ratio $\frac{Q_{LA}^{O_2}}{Q_{O_2}}$. This factor demonstrates the amount of lactic acid produced in spite of respiration and, therefore, indicates the proportion between oxidative and glycolytic carbohydrate fission. The latter factor proved especially useful for the definition of the carbohydrate metabolism of neoplasms.

As mentioned before, normal tissues, in general, react in such manner that an appreciable glycolysis occurs only under anaerobic conditions, whereas the presence of oxygen very markedly lowers the glycolysis, or causes it to disappear completely. The intensity of anaerobic glycolysis varies for individual tissues and reaches very high values in rapidly growing ones, such as in embryonic and neoplastic tissues. For instance, the figure $Q_{LA}^{N_2}$ in tumors averages 12, in muscle 0.1; that is, within an hour, the tumor forms a quantity of lactic acid approximately corresponding to 12 per cent of its weight. When oxygen is permitted to be present—and this has been a fundamental disclosure—the glycolysis of muscle disappears almost completely, and, what is still more striking, the same occurs in fetal tissue with strong anaerobic glycolytic activity, whereas sixty to eighty per cent of the glycolysis of malignant tumors remains in force. Hence, tumors produce lactic acid under aerobic conditions and this metabolic peculiarity represents, according to Warburg, a characteristic quality of neoplastic growth while, as remarked, a strong anaerobic glycolysis is common to all tissues with a high growth rate, therefore, also to embryonic tissue. Significant additional information regarding tumor metabolism has been derived from the analysis of the above enumerated ratios. As to the Meyerhof ratio—

$$\frac{Q^{N_2}_{LA} - Q^{O_2}_{LA}}{Q_{O_2}}$$

—according to Warburg the metabolism of neoplastic tissue does not deviate essentially from that of normal tissue. The figure varies for tumors between one and two in the order of magnitude found by Meyerhof for muscles. This indicates that one molecule of oxygen consumed causes the disappearance of one to two molecules of lactic acid. Since one molecule of oxygen can accomplish the combustion of one-third of a molecule of lactic acid, the molecules of lactic acid may be substituted for the molecules of oxygen in the denominator of the aforementioned fraction, thus leading to the ratio: $\frac{\text{Molecules of lactic acid made to disappear}}{\text{Molecules of lactic acid oxidized}}$; or in other words, this ratio is obtained by multiplying the Meyerhof quotient by three. Thus the analysis of the Meyerhof quotient did not reveal to Warburg an anomaly of the tumor metabolism, but, to quote him again, "the numerical linkage between extent of respiration and effect of respiration is the same." It must be mentioned that, in contrast to this statement, another investigator observed a considerable lowering of the Meyerhof quotient in tumor tissue, a phenomenon which he explained not so much by the increased glycolytic capacity but more by a disturbance of the carbohydrate resynthesis.[94] There will be occasion to refer to additional work dealing with this problem when the Pasteur effect in tumors is discussed.

An entirely different aspect is exhibited by the ratio $\frac{\text{aerobic glycolysis}}{\text{respiration}}$, (Warburg's ratio), which expresses the proportion between oxidation and fermentation. A value of four has been thus obtained for rat carcinomas. The original ratio, containing milligrams of lactic acid in the enumerator and mols of oxygen in the denominator, can be modified by expressing both the glycolysis and the respiration in mols of consumed glucose. Since the lactic acid in the enumerator corresponds to one-half of a mol of glucose, and the oxygen in the denominator to one-sixth of a mol of glucose, the original ratio of $\frac{4 \text{ (lactic acid)}}{1 \text{ (oxygen)}}$ is converted into $\frac{2}{1/6}$ or $\frac{12}{1}$, which means that "out of thirteen glucose molecules attacked, one is oxidized and the remainder fermented (glycolyzed); hence, the metabolism of carcinomatous tissue in the presence of oxygen is predominantly a fermentative metabolism."

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[94] R. Bierich: Internat. Conf. Cancer, London, p. 61 (1928).

On the other hand, the aerobic metabolism of normal tissue is pre-eminently based on oxidation; in the kidney, the pancreas, the liver and some other organs, the ratio $\frac{\text{aerobic glycolysis}}{\text{respiration}}$ is approximately zero. Certain proliferating tissues, such as epithelia of gonads, thymus and lymph nodes, show higher values, averaging 0.66, but these values are still far below those of neoplastic tissue. It is of interest to point out that fetal tissue, in spite of its high growth intensity and corresponding large anaerobic formation of lactic acid, exhibits a predominantly oxidative metabolism as expressed in a ratio of only 0.1; or, in other words, out of ten glucose molecules utilized by embryonic tissue in the presence of oxygen, only one is glycolyzed, while nine are oxidized. Benign tumors occupy an intermediate position between the metabolism of normal tissues and that of malignant tumors. Warburg's investigations, extending to nasal polyps and papillomas of the urinary bladder, for these tissues show ratios of 0.5 to 1.45, or an average of 1.0. Hence, "the proportion $\frac{\text{glycolytic metabolism}}{\text{oxidative metabolism}}$ is shifted considerably to the form of oxidative metabolism" when compared with the behavior of malignant tumors. Nevertheless, the metabolic deviation of benign tumors from the reaction of normal tissues may be connected with the clinical observation that some of these benign tumors, particularly of the bladder and of the intestines, not too rarely undergo malignant transformations.

It may be remarked that the results outlined were obtained at first with Flexner-Jobling rat carcinomas serving as an example of neoplastic tissue, but continuation of this work yielded confirmation of the above mentioned findings by means of testing various animal and human neoplasms.[91] It is also noteworthy that in some of these experiments practically identical results were obtained when chemical methods were employed instead of manometric ones for the determination of the lactic acid.[91]

On the basis of these discoveries and in connection with Meyerhof's concepts of the influence of respiration on glycolysis, Warburg was led to the following consideration: When a tissue glycolyzes under aerobic conditions, this—he surmised—indicates that the respiration is insufficient to "master the flood of lactic acid" (Meyerhof). Hence, Warburg concluded that a disproportion between respiration and glycolysis exists in malignant tumors. For a clarification of this problem, he attempted to create a "tumor metabolism" artificially in a normal tissue. Such experiments necessitated a damage to the respiration and this was accomplished by treatment of the tissue with prussic acid, or by storing it for a longer period of time in the absence of oxygen. Actually, such experimental conditions brought about a change in the metabolism of embryonic tissue, making it similar to that of neoplasms. Advancing one step further, Warburg assumed that these results might be utilized to establish a theory of tumor development based on metabolic alterations; that is, he conceived the possibility that impairments of cellular respiration, whether caused by arteriosclerosis or by other forms of chronic oxygen deficiency, may not lead to death in a number of cells, but instead to a change of their metabolism in a direction which has been described above as being typical for malignant cells. In other words, a selection of cells might take place which, a priori, are not dependent on oxygen supply for their existence, but which are endowed with the property of using glycolytic processes for covering their energy requirements. However, the general validity of this conception which regards an oxygen deficiency as a causative factor for the development of neoplasia is subject to doubts. As far as clinical observations are concerned, it has been reported that autopsies revealed a rarer incidence of arteriosclerosis in tumor patients than in persons deceased from other diseases, a circumstance which it was believed might indicate that different constitutional types possess a tendency to neoplasia, or to arteriosclerosis, respectively.[95] In any case this statement disproves the assumption that an impairment of cellular respiration, such as may be caused by arteriosclerosis, predisposes persons to the formation of malignant tumors. Another author proposed that a narrow circumference of the aorta, as measured roentgenologically and compared with standard values, should be considered as an indication of a predisposition to cancer, since this anatomical deviation from the normal may cause an insufficient oxygen supply and a reduction of oxidative processes in the body.[96] The speculative character of this argument is obvious, and the objection may be raised that by way of such reasoning cardiac defects ought likewise to be considered as diseases predisposing patients to neoplasia, an assumption by no means supported by clinical experience. Finally, reference is made to animal experiments in which lack of oxygen was supposedly demonstrated to be a factor contributing to tumor development. For this purpose an oxygen deficiency in

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95. E. Schneider: Med. Klin. *32*: 1565 (1936).
96. S. Kreuzfuchs: Wien. klin. Wchnschr. *51*: 218 (1938).

organs of mice was created by means of ligation of the artery of one or both uterine horns, an interference which in some instances was reported to be followed by the formation of neoplasms.[97] But since only one of these tumors developed in the uterus, while all others developed in the breast of the animals, these results cannot be considered as convincing evidence for a tumorigenic effect of the restriction of the oxygen supply.

In addition to these attempts—so far unsuccessful—to base the tumor etiology on metabolic alterations, Warburg pointed out certain aspects of the tumor metabolism, which might be utilized for purposes of cancer therapy. In this connection, the author and his collaborator[90] demonstrated that tumor cells are capable of surviving a lack of oxygen, and can retain their viability under these conditions. In such experiments tumor slices (Flexner-Jobling rat carcinoma, Jensen rat sarcoma, Rous sarcoma of fowl) were kept in a mixture of nitrogen and carbon dioxide in a Ringer solution, which either contained glucose or was free of sugar. At the end of the experiment the vitality of the cells was judged, both by the possibility of their growth after being inoculated into animals, and by the determination of their metabolism. It was observed that "tumor cells which are kept anaerobically in glucose-free Ringer solution do not live after 24 hours, whereas lack of oxygen can be tolerated for 24 hours (in the case of Jensen sarcoma for 72 hours), when the Ringer solution contains glucose." Hence, for the destruction of tumor cells, it is necessary to prevent both glycolysis and respiration; that is, to employ a medium free of glucose and of oxygen. Such conditions cause the death of tumor cells even after four hours. Although these results do not appear encouraging as far as the possibility of clinical cancer treatment is concerned, Warburg nevertheless believed that the aspects outlined provide an interesting insight into the conditions of neoplasms in vivo. He stressed the fact that the supplying of tumors with glucose and oxygen via the bloodstream is greatly inferior to that of normal tissue and, accordingly, he assumed that restrictions of the arterial oxygen and glucose supply may prove detrimental to neoplastic tissue, although deficiencies of the same extent may not impair the vitality of normal tissue. Because of the poor vascularity, tumor cells are, so to speak, constantly at the limit of their vitality and, accordingly, may be affected by restrictions of a lesser degree. It has previously been mentioned that malignant tumors consume a large proportion of the carbohydrates supplied to them by the blood stream, and investigations of Warburg and his collaborators[98] on this subject yielded the following results. Perfusion of tumors with arterial blood is followed by a reduction of the glucose concentration averaging fifty-seven per cent, whereas in normal tissues the reduction ranges between two and eighteen per cent. Determinations of the concentration of lactic acid showed that no production of this substance takes place after perfusion of normal organs, whereas tumor tissues caused a formation of forty-six to forty-nine milligrams of lactic acid per 100 c. c. On the basis of these figures, Warburg calculated the oxygen requirements of tumors and found that the combustion of glucose, which is oxidized in tumors, required as much oxygen as was provided by the blood supply. In view of this narrow oxygen limit, it is reasonable to conclude that even small oxygen restrictions lead to a deficit. Since, however, tumor cells are not confined to oxidative metabolism, but have, so to speak, the choice between aerobic and anaerobic carbohydrate utilization, a decisive damage of tumor cells can only be expected from a simultaneous prevention of respiration (oxygen deficiency) and of glycolysis

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[97] J. E. Davis: Canad. M. A. J. 36: 237 (1937).

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[98] O. Warburg, F. Wind & E. Negelein: Klin. Wchnschr. *5*: 829 (1926).

(glucose deficiency). It is obvious that practical results for the treatment of human neoplasia can hardly be derived from these facts, yet animal experiments performed by Warburg led to the following significant results. When tumor-bearing rats were kept for forty hours in gas mixtures containing only five per cent by volume of oxygen, the determination of the tumor metabolism performed after this period showed that the majority of the tumor cells had been killed, since neither respiration nor glycolysis was detectable. According to Warburg, a similar effect may be involved in therapeutic experiments in which a lowering of the blood sugar level was achieved by the administration of insulin to cancer patients, a treatment discussed in chapter VII. Numerous experiments, in which a depression of the excessive tumor glycolysis by means of various chemical compounds was attempted, will be discussed later.

Later work on carbohydrate metabolism of neoplasms. The experimental data, and the theoretical conclusions drawn from them, which have been described, have formed the subject of continued examination and elaboration.[cf. 46, 47, 99] In its essentials the validity of Warburg's work has been completely corroborated, and claims of priority have even been advanced by Bierich.[100, cf. 101] It must be emphasized that identical results were obtained by employing chemical methods[102] and also by the manometric methods used by Warburg.[103-107] The basic significance of Warburg's work has not been changed by investigations, in which certain

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99. D. Burk: Occas. Pub. Am. Ass. Advancement Sc. *4*: 121 (1937).
100. R. Bierich: Klin. Wchnschr. *6*: 2047; 2288 (1927).
101. O. Warburg: Klin. Wchnschr. *6*: 2287 (1927).
102. A. Mahnert: Wien. klin. Wchnschr. *37*: 1114 (1924).
103. P. Rona & W. Deutsch: Klin. Wchnschr. *5*: 1216 (1926).
104. A. Loeser: Klin. Wchnschr. *6*: 587 (1927).
105. H. G. Crabtree: Biochem. J. *23*: 536 (1929).
106. F. Dickens & F. Šimer: Biochem. J. *24*: 1301 (1930); *25*: 985 (1931); Lancet *2*: 10 (1930).
107. F. Dickens & G. D. Greville: Biochem. J. *26*: 1546 (1932); *27*: 1134 (1933).

mouse tumors were found to exhibit an aerobic glycolysis of lesser extent [108–110] than was observed in the original work. The only definitely controversial statements reported [111] have been explained by Warburg [112] as depending on methodological inadequacies. It is easily understandable that in tumor slices, tested for their metabolic properties, the admixture of stroma, of necrotic material, of nonmalignant tissue or of leucocytes must be carefully considered and estimated by histological examination, since otherwise a comparison of various tumors containing different quantities of such impurities is not feasible. Such precautionary measures were actually taken by Warburg and by most of the later investigators. Additional proof for the necessity of combined histological and metabolic analyses has been furnished by an extensive study of recent date dealing with the carbohydrate metabolism of various fowl tumors.[113] This work confirmed and amplified the older data on the characteristic aspects of tumor metabolism, inasmuch as the tumors tested in general exhibited a high anaerobic and aerobic glycolysis, as determined by manometric methods, or by chemical estimation of the formation of lactic acid. It must be stressed that the metabolic properties were found to be much the same in filterable fowl tumors (virus tumors) and in fowl tumors which had been chemically induced with methylcholanthrene and which were not transferable by a filterable agent. Interesting results regarding the oxygen consumption, the respiratory quotient and the Pasteur effect, which were obtained

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[108] F. Dickens & D. H. Patey: Lancet *2*: 1229 (1930).

[109] J. B. Murphy & J. A. Hawkins: J. Gener. Physiol. *8*: 115 (1925).

[110] J. A. Hawkins: J. Gener. Physiol. *9*: 771 (1926).

[111] R. Bauer & W. Nyiri: Wien. klin. Wchnschr. *38*: 853; 881; 1188 (1925).

[112] O. Warburg: Wien. klin. Wchnschr. *38*: 1188 (1925); Klin. Wchnschr. *4*: 2396 (1925).

[113] D. Burk, H. Sprince, J. M. Spangler, E. A. Kabat, J. Furth & A. Claude: J. Nat. Cancer Inst. *2*: 201 (1941).

in this study, will be considered in later sections of this chapter. Because of the lesser probability of a participation of impurities, particular interest may be ascribed to metabolic studies performed with neoplastic tissue cultures, studies which soon after Warburg's original announcement confirmed the existence of the high glycolytic activity of malignant tumors.[114, 115] More recently, such work has been carried out with tissue cultures of Jensen rat sarcomas,[116] and corroborative evidence for the metabolic characteristics of neoplasms was also obtained with pure cultures of Walker rat sarcoma 319.[117] The metabolism of the latter tumor was determined by other workers, who employed a specially devised apparatus for measurements of the metabolic activity of growing tissue cultures, and who compared the data obtained with similar results recorded in the literature.[27] The fact that neoplastic tissue cultures in general—i.e., tumor tissues growing outside of the body—exhibit a metabolic behavior very similar to that of tumors removed from the body, strongly suggests that the qualities described are inherent in the tumor cells proper and, accordingly, represent one of the few instances in which biochemical methods have so far been able to characterize malignant tumors in a nearly specific manner. To what extent this assumption is still tenable in the light of seemingly contradictory evidence will be shown later.

In this connection, a very important point is brought up by the question as to whether and at what period metabolic alterations, similar to those described for malignant tumors, occur in tissues exposed to carcinogenic stimuli. Investigations of this type have only infrequently been reported and,

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114. F. Wind: Klin. Wchnschr. *5*: 1355 (1926).
115. F. Demuth & R. Meier: Biochem. Ztschr. *212*: 399 (1929).
116. B. Snellman: Arch. f. exper. Zellforsch. *20*: 213 (1937).
117. J. Victor & W. H. Lewis: Am. J. Cancer *29*: 503 (1937).

accordingly, a conclusive answer can be expected only from continued work. Controversial statements have been made as to the effect of the feeding of butter yellow on the liver metabolism of rats. On the one hand, it was reported that prior to the formation of malignant hepatomas a marked increase of the anaerobic glycolysis was observed, followed by an increase of the aerobic glycolysis when hepatomas developed.[118] On the other hand, other workers have stated that no increase of the anaerobic glycolysis takes place in precancerous livers of rats fed with butter yellow; the formation of neoplastic tissue was said to be accompanied by a sudden change of the metabolism, expressed in a high glycolytic activity.[119] The fact that hepatic rat tumors, induced by butter yellow, possess the characteristic metabolic properties of neoplasms has been established, and it has also been shown that the metabolism of these tumors differs markedly from that of regenerating liver tissue.[120] Studies, in which the oxygen consumption of precancerous tissue was determined, and the theoretical conclusions derived from this work, will be discussed in the last section.

Kinetics of tumor glycolysis. The details of Warburg's results regarding the glycolysis of Flexner-Jobling rat carcinoma may be supplemented here. The glycolysis increased to a certain extent with the pH and with the concentration of bicarbonate; it was augmented with an increasing glucose concentration reaching a maximum at a glucose concentration of 0.2 to 0.3 per cent in the medium; 37° C proved to be the optimal temperature. In addition to glucose, tumor slices also glycolyzed mannose, fructose, and galactose, the rate of glycolysis diminishing in the order of the sugars named.[88] It was later found that anaerobic formation of lactic acid from fructose occurs only in the liver, among normal organs, but this property was inherent in Jensen rat sarcoma, in fibrosarcomas of fowl and, to a lesser extent, also in the Rous fowl sarcomas, but not in Brown-Pearce rabbit carcinomas or in two different mouse tumor strains.[121] Transplanted mouse tumors have been tested as to their ability to glycolyze various hexoses and the following results were obtained. When the lactic acid production from glucose was put down as 100, mannose yielded an approximately equal glycolysis, whereas the value for fructose was determined as 12, and for galactose as 10. Pentoses were scarcely glycolyzed; among trioses, dihydroxyacetone showed a glycolysis of 50, and glyceric aldehyde of 32. Of hexose-derivatives, compounds of pyranose (1, 5-glucose) and furanose (1, 4-glucose) were poorly utilized, the γ-glucose (Pringsheim's 1, 6-glucose) not at all. It was stressed that the degree of utilization of the various sugars by tumor tissue may not only depend on the presence of specific enzymes, but that a different permeability of the tumor cell membranes for the sugars must also be considered.[122] In tissue cultures of Jensen rat sarcoma, a substitution of glucose by fructose or galactose lowered the glycolysis by forty to seventy per cent, but it is noteworthy that this effect was not accompanied by a change of the rate of growth.[116] Walker rat sarcoma 319 was found to glycolyze glucose, dihydroxyacetone and hydroxypyruvic acid, as determined by a decrease of the pH in the tumors in vivo and by estimation of the lactic acid content of removed tumors.[123] Rat hepatomas induced by butter yellow were found to attack glucose, but not glycogen, which latter substance seemed to be absent in the tumors.[119] The utilization of hexose phosphoric esters by tumors will be considered in a later section. Reference is made to the statement that the optimum of the pH for glycolysis is shifted to the alkaline side in tumors (7.8), as compared with muscle (7.5).[124] It is of interest that, to a certain extent, the tumor metabolism—especially the glycolysis—appears to be independent of the medium employed; this is indicated by the fact that the same metabolic phenomena are observed in tumor slices tested in Ringer solution containing glucose, or in serum. As will be shown later, serum proved to be the only adequate medium for a number of normal tissues. Hence it is not surprising that no essential difference was found in the metabolism of tumor tissue suspended in the serum of normal or of cancerous persons.[125] If these findings appear to be in conflict with the aspects of the carcinolytic reaction (cf. chapter X), in the writers' opinion, the two phenomena under discussion are by no means comparable. The carcinolytic reaction concerns dead cancer cells, the lysis of which by normal serum is the expression of the presence of defensive agents present in the healthy body. On the other hand, the metabolic qualities of neoplastic tissues are indicative of their vitality, which, as is generally known, is strong enough to result in tumor formation when small tumor fragments are inoculated into a healthy animal. A somewhat different problem is involved in the observation that rat tumors showed a decreased aerobic glycolysis when suspended in inflammatory exudates (cantharides vesicles), as compared with the glycolysis of the same tumor contained in serum.[126] Whether this result justifies the assumption that inflammatory processes counteract tumor growth (cf. chapter VIII) by means of an alteration of the tumor metabolism cannot be decided on the basis of the meagre experimental data available.

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[118] M. Nakatani, K. Nakano & Y. Ohara: Gann *32*: 240 (1938).

[119] J. W. Orr & L. H. Stickland: Biochem. J. *35*: 479 (1941).

[120] D. Burk, O. K. Behrens & K. Sugiura: Cancer Research *1*: 733 (1941).

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[121] F. Dickens & F. Šimer: Biochem. J. *26*: 1546 (1932).

[122] O. O. Meyer, C. McTiernan & W. T. Salter: Am. J. Cancer *22*: 76 (1934).

[123] F. F. Beck, R. Musser, C. J. Carr & J. C. Krantz, Jr.: Am. J. Cancer *32*: 434 (1938).

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[124] E. Boyland & C. A. Mawson: Biochem. J. *28*: 1409 (1934).
[125] H. A. Krebs & F. Kubowitz: Biochem. Ztschr. *189*: 194 (1927).
[126] R. Lohmann: Klin. Wchnschr. *10*: 1799 (1931).

Several studies have dealt with the problem as to whether or not the tumor glycolysis is inseparably linked with the cellular structure of neoplasms. A considerable reduction of the sugar concentration was found after incubation of sterile cell-free filtrates of transplanted mouse carcinomas and sarcomas,[127] but no tests were made as to whether this loss of sugar was accompanied by a production of lactic acid and, accordingly, these results are not conclusive. A close linking of the tumor glycolysis with cellular structure was suggested by a study, according to which grinding of tumor tissue resulted in a diminished glycolysis, and freezing or extraction caused complete loss of the glycolytic activity of tumor substrates.[128] These findings are in marked contrast to those pertaining to glycolysis of muscle, an organ from which extracts with high glycolytic activity can be prepared according to the method of Lohmann. Other workers also failed to notice any formation of lactic acid in tumor extracts,[129] and the report that powdered Rous sarcoma substrate was found capable of glycolyzing glucose [130] does not conflict with the earlier statements, since this fowl tumor can also be transmitted by substrates, the cellular structure of which has been destroyed. A far-reaching elucidation of the reason as to why tumor extracts, prepared according to the ordinary methods, do not glycolyze was accomplished by later work. It was shown that extracts, prepared from mouse sarcomas in a manner corresponding to Lohmann's muscle extract, failed to produce lactic acid from added glycogen, glucose or fructose. These findings are in satisfactory agreement with the previous reports. Yet glycolysis occurred in the extracts

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127. N. Alders, H. Chiari & D. Laszlo: Biochem. Ztschr. *180*: 46 (1927).
128. D. P. Barr, E. Ronzoni & J. Glaser: J. Biol. Chem. *80*: 331 (1928).
129. H. Kraut & E. Bumm: Ztschr. f. physiol. Chem. *177*: 125 (1928).
130. L. Karczag: Biochem. Ztschr. *230*: 421 (1930).

when carbohydrates were added in the form of hexose monophosphate or hexose diphosphate.[131] According to these and to previous results, tumor extracts, as well as tumor slices, are unable to utilize glycogen—in contrast to muscle extracts —but do glycolyze hexosephosphates. This was tentatively interpreted as indicating a disturbance of the process of carbohydrate phosphorylation, a problem considered in the next section. However, the conditions under which tumor extracts are capable of glycolysis were demonstrated as being principally dependent on another factor; that is, the addition of adenylpyrophosphate, viz., a coenzyme of glycolysis, and of yeast cozymase (coenzyme I) to cell-free mouse sarcoma extracts was shown to result in a glycolysis in the extracts, which equalled that of tumor tissue, regardless of whether glucose, hexosemonophosphate or hexosediphosphate served as substrate. Cozymase and adenylic acid were found to be rapidly destroyed by the tumor extracts, and this apparently is the reason why no lactic acid is produced by tumor extracts without the addition of these substances which are required for the glycolytic process.[132] The studies mentioned considerably clarified some of the aspects of tumor glycolysis, and the possibility for using homogeneous extracts of mammalian tumors for metabolic determinations may prove valuable. Lesser significance may, for the present, be ascribed to experiments in which the glycolysis of alcoholic extracts was determined. Such extracts of malignant tumors, mixed with glucose solution, were reported to produce larger amounts of lactic acid than similar extracts of benign tumors.[133]

Several authors considered whether the high glycolytic

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131. F. H. Scharles, M. D. Baker & W. T. Salter: Am. J. Cancer *25*: 122 (1935).

132. E. Boyland, M. E. Boyland & G. D. Greville: Biochem. J. *31*: 461 (1937).

133. M. Novi: Clin. chir. *36*: 629 (1933); abstr. Am. J. Cancer *23*: 844 (1935).

activity of neoplasms is linked with the presence of substances which may activate the glycolysis of normal tissues. Some investigators arrived at negative conclusions,[134, 135] but others reported an abundant presence of a coferment in tumor extracts, which activated the glycolysis.[136, 137] These discrepancies may have been due to the different experimental arrangements used. Positive results were claimed with tumor extracts which were found to cause an increase of the glycolysis of kidney tissue of ten to twenty times.[138] The coferment in question was also obtained in the form of a dry powder from tumors; it was characterized by a high activity, and by thermolability, which latter property clearly distinguishes it from the cozymase of yeast fermentation.[129, 139] This substance, called coferment T, was originally believed to be pyruvic acid,[140] but this view was abandoned on the basis of later work.[141] Most of the later studies supported the assumption that tumors contain activators of glycolysis. An increased glycolysis of muscle extracts was achieved by the addition of boiled tumor extracts;[142] substances which were precipitated by alcohol from tumors treated with acetone increased the glycolysis of liver tissue;[143] and sera of tumor patients were also reported to contain an agent capable of activating glycolysis.[144] Methyl alcoholic extracts of human neoplasms or of rat tumors, of sera or of erythrocytes of

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[134] J. Brooks & M. Jowett: Biochem. J. *22*: 1413 (1928).

[135] F. Pentimalli: Biochem. Ztschr. *242*: 233 (1931).

[136] C. Neuberg & A. Gottschalk: Biochem. Ztschr. *154*: 492 (1924).

[137] H. von Euler & K. Myrbäck: Pflüger's Arch. f. Physiol. *210*: 521 (1925).

[138] N. Waterman: Arch. neerl. physiol. *9*: 573 (1924); Bull. Assoc. franç. p. l'étude du cancer *13*: 396 (1924).

[139] H. Kraut & E. Bumm: Ztschr. f. physiol. Chem. *184*: 196 (1929).

[140] B. Mendel: Klin. Wchnschr. *10*: 118 (1931).

[141] H. Kraut & R. W.-Nefflen: Ztschr. f. physiol. Chem. *232*: 270 (1935).

[142] C. Frisch & R. Willheim: Biochem. Ztschr. *272*: 332 (1934).

[143] W. M. Rubel & W. A. Belitzer: Acta Cancrol. *1*: 317 (1935).

[144] G. Landegger & H. Pirker: Ztschr. f. Immunitätsforsch. u. exper. Ther. *55*: 358 (1928).

tumor patients, were all found to increase the glycolysis of normal red blood cells, whereas analogous extracts prepared from normal tissues failed to do so.[145] Boiled tumor extracts were shown to increase the fermentation of washed dry yeast and were, accordingly, assumed to contain cozymase.[132] A special type of glycolysis activation described as "extra-fermentation" ("extra-glycolysis") is represented by the phenomenon that an increased glycolysis occurs in tissues which, prior to the test, are kept in anaerobiosis.[146] It was assumed that this effect was caused by an accumulation, during the anaerobic phase, of substances stimulating glycolysis, and it is noteworthy that tumor tissue also exhibited a markedly increased glycolysis under such conditions.[147]

Enzymes which participate in intermediate steps of glycolysis were studied in connection with the carbohydrate metabolism of neoplastic tissues. A substance was isolated from tissues treated with acetone, which decomposed hexose diphosphoric esters to methylglyoxal, and it was reported that malignant tumors yielded particularly large amounts of this glycolase.[148] The glyoxalase activity of malignant tumors has been described in somewhat conflicting ways; rat tumors were reported to possess an enzyme activity corresponding approximately to that of normal muscle,[149] or even to contain lesser amounts of this enzyme.[150] On the other hand, a changed activation of methylglyoxalase in tumors may be considered; that is, the oxygen deficiency prevailing in neoplastic tissues has been assumed to cause an increased cleavage of glutathione to cysteine, which latter compound repre-

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145. M. Ascoli: Libro de Oro, Buenos Aires, p. 52 (1935).
146. O. Rosenthal & A. Lasnitzki: Biochem. Ztschr. *196*: 340 (1928).
147. O. Rosenthal: Ztschr. f. Krebsforsch. *32*: 220 (1930).
148. C. Neuberg, M. Kobel & H. Laser: Ztschr. f. Krebsforsch. *32*: 92 (1930).
149. M. Jowett & J. H. Quastel: Biochem. J. *28*: 162 (1934).
150. M. E. Platt & E. F. Schroeder: J. Biol. Chem. *106*: 179 (1934).

sents a potent activator of methylglyoxalase. The increased conversion of methylglyoxal to lactic acid which thus may be induced by the activated enzyme was believed to be responsible for the accumulation of lactic acid in neoplasms, and the rapid disappearance of methylglyoxal was thought to interfere with the re-synthesis of this compound to hexose (Pasteur-Meyerhof effect, cf. later).[151] In agreement with the aforementioned views, the glyoxalase activity of tumors, measured under anaerobic conditions, was found to be very high. A different effect on glycolysis and on glyoxalase activity was observed as resulting from roentgen irradiation at low temperatures. That is, in spite of an interference with the glycolysis, the glyoxalase activity was practically unimpaired.[152] Another partial enzyme of carbohydrate cleavage, viz., the zymohexase which splits the 6-carbon-chain of hexose phosphates into 3-carbon-compounds (dihydroxyacetone phosphate), has been found to exhibit an activity in tumors corresponding approximately to one-tenth of the activity found in muscle extracts.[153] An enzyme which converts phosphoglyceric acid into pyruvic acid and phosphoric acid was demonstrated to be present in breast tissue of lactating cows and in human breast cancer among other tissues. The amount of pyruvic acid formed in the breast cancer was found to exceed that of the phosphoric acid, the reason probably being a rapid utilization of the phosphoric acid for other intermediate syntheses.[154] Interesting information has been furnished as to the cozymase (coenzyme I) content of malignant tumors. In one of these studies, it was ascertained that the ratio $\frac{\text{Reduced cozymase (dihydro-cozymase)}}{\text{cozymase}}$ was considerably increased in Jensen rat sarcomas, ranging between four and ten, whereas this ratio was found in normal tissues and also in fetal tissues below or around unity.[155] This increase of dihydrocozymase in the tumors must be considered as a consequence of the lack of specific dehydrogenases, a problem for which supplementary data will be found in the section on respiration. The absolute values for coenzymes I and II were determined in Walker carcinoma 256 as being about one-sixth of that of normal adult rat tissues; low concentrations of cozymase were also found in fetal rat liver, but the values in this organ rose rapidly after birth. Only traces of cozymase were detected in three human cancers tested.[156] In this connection it is of great interest to refer to studies dealing with the hepatoma formation induced in rats by means of feeding butter yellow. During the period of the administration of the carcinogen, a progressive decrease of the content of coenzyme I was observed in the livers of the animals, and the hepatomas, eventually developing, exhibited extremely low values.[157] Vitro experiments furnished results which clarified the mechanism by means of which the carcinogen interferes with an important phase of the mettabolism; that is, addition of metabolites of butter yellow—viz., p-phenylenediamine and N, N-dimethyl-p-phenylenediamine—was shown strongly to inhibit the coenzyme activity in fermenting yeast. Butter yellow itself did not exert such an effect, and acetylation of the mentioned metabolites of butter yellow cancelled their inhibiting action on coenzyme.[157a] As stated in chapter II, such an acetylation was demonstrated as taking place in the body of animals receiving butter yellow, and hence it may be regarded as a process of detoxication.

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[151] R. Bierich & A. Rosenbohm: Ztschr. f. physiol. Chem. *223*: 136 (1934); *231*: 39 (1935).

[152] H. G. Crabtree: Biochem. J. *30*: 1622 (1936).

[153] E. Boyland & M. E. Boyland: Biochem. J. *29*: 1910 (1935).

[154] C. Antoniani & P. Bucalossi: Tumori n.s. *10*: 375 (1936).

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[155] H. von Euler, M. Malmberg & G. Günther: Ztschr. f. Krebsforsch. *45*: 425 (1937).

[156] F. Bernheim & A. V. Felsovany: Science *91*: 76 (1940).

[157] C. J. Kensler, K. Sugiura & C. P. Rhoads: Science *91*: 623 (1940).

[157a] C. J. Kensler, S. O. Dexter & C. P. Rhoads: Cancer Research *2*: 1 (1942).

Phosporylation. There has been some doubt as to whether phosphorylation is an essential step in the intermediate carbohydrate metabolism of malignant tumors as is the case in muscle. In addition, several investigations have been concerned with comparing this process in muscle—which has been very well defined—with corresponding phases of the tumor metabolism. Negative opinions as to the occurrence of phosphorylation in neoplastic tissues were based, in part, on the observation that a pancreas extract. which prevented the glycolysis of muscle by means of inhibiting the phosphorylation, did not affect the tumor glycolysis.[128] Other workers stressed that hexose phosphoric esters are either very slowly glycolyzed by tumors, or not at all,[124, 147, 158, 159] but these statements are open to some objections. In particular, it must be considered that tumor metabolism may involve other hexose phosphoric esters than those tested [147] and, furthermore, the previously enumerated experiments showed that under special conditions tumor substrates are capable of forming lactic acid from hexose phosphates.[131, 132] An inhibition of tumor glycolysis has been observed after the addition of iodoacetic acid,[160] and this effect was recognized as being dependent on a suppression of phosphorylation. Finally, more direct evidence was furnished for the occurrence of phosphorylation in the course of tumor metabolism. The aerobic glycolysis of mouse tumors was shown to be accompanied by a decrease of the inorganic phosphate fraction and an increase of the acid-soluble organic phosphorus;[161, 162] the presence of hexose phosphoric esters—

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158. R. O. Loebel: Biochem. Ztschr. *161*: 219 (1925).
159. S. T. Harrison & E. Mellanby: Biochem. J. *24*: 141 (1930).
160. E. Lundsgaard: Biochem. Ztschr. *220*: 8 (1930).
161. R. Willheim & J. Schmerler: Biochem. Ztschr. *254*: 355 (1932).
162. W. R. Franks: J. Physiol. *74*: 195 (1932).

lactacidogen—was reported for Ehrlich mouse carcinomas.[163] The concentration of hexose phosphates was increased after suppression of the tumor glycolysis by means of sodium fluoride.[164] Continued studies showed that the lactacidogen values in Ehrlich mouse carcinomas were inversely proportional to the concentration of lactic acid. It was assumed that the tumors contain both hexose monophosphates and hexose diphosphates, but the experimental data suggested the probability that the glycolysis of tumors involves hexose phosphates which are different from those occurring in the glycolysis of muscle.[165] Similar conclusions were drawn by other workers from the observation that tumor extracts—in contrast to muscle extracts—were incapable of catalyzing the reaction between triose phosphates and pyruvate, and that incubation of tumor extracts mixed with glycogen, sodium fluoride and phosphates yielded products differing from those obtained in similar experiments employing muscle extracts.[166] Accordingly, it appears very probable that tumor glycolysis and muscle glycolysis function differently, but during tumor glycolysis phosphorylation also appears to take place, although it cannot as yet be decided whether this is an indispensable intermediate stage in the breakdown of carbohydrates in neoplasms.

Carbohydrate metabolism of healthy organs of tumor hosts. It is understandable that the extension of the studies of metabolism to organs of tumor hosts, organs, which are morphologically free of neoplastic growth, should be of interest because, depending on the results obtained from such investigations, an answer might be found to the question as to whether the metabolic alteration in neoplasia is confined to the malignant tumors proper or, in addition, possesses a systemic character. Several reports were presented in support of the latter assumption. Six days after the inoculation of tumors into mice, their livers and kidneys, though free of metastases, showed an increased aerobic glycolysis.[167] A particularly high glycolysis was observed in the spleens of tumor-bearing rats.[168] Likewise, another author disclosed findings of an augmented glycolysis of tumor-free organs (muscle, liver, kidney) of mice with Ehrlich carcinoma.[169] A number of investigations have dealt with the glycolysis of blood, a substrate also accessible to clinical examinations. An increased glycolysis of venous blood draining the tumor-bearing wing (Rous sarcoma) of fowl was observed,[170] but more valuable information may be expected from experiments which employed blood obtained from regions removed from the site of the tumor. The results of this work, however, are far from uniform, and so far do not permit the definite ascribing of a metabolic quality definitely deviating from the normal to erythrocytes of tumor hosts. On the one hand, a group of authors claimed they had found that the red blood cells of cancerous patients, or of rats with transplanted tumors, formed twice to six times as great an amount of lactic acid as normal erythrocytes,[171] a phenomenon which they believed to be constant enough to warrant its diagnostic utilization (cf. chapter X). In further support of the conception that the glycolysis increase is directly connected with the tumor development, it was pointed out that erythrocytes of rabbits,[172] or of rats,[173] exhibited an increased glycolysis after intraperitoneal injection of tar or of benzpyrene into the animals. Red blood cells of tumor hosts were also reported to exhibit an increased anaerobic glycolysis [174] and to produce lactic acid from fructose and mannose.[175] Other authors, however, arrived at different results, inasmuch as they found the glycolysis of erythrocytes of tumor patients to show great variations,[176] or to be increased only in approximately seventy-seven per cent of the cases tested,[177] while some workers failed to notice any significant difference between the lactic acid quantities formed by erythrocytes of normal or of cancerous persons.[178] These discrepancies clearly indicate that the glycolysis of red blood cells in neoplasia undergoes considerable variations during the course of the disease, and this view is strongly supported by a recent analysis of these relations. The glycolytic activity of erythrocytes of tumor-bearing mice and rats showed an early increase, then an abrupt fall, followed by a subsequent rise and a renewed drop coinciding with the onset of cachexia.[179] Hence, it appears plausible to assume that the results of estimations of blood glycolysis in human neoplasia will largely depend on the stage of the disease in the cases examined. Studies which were concerned with the effects of colloidal carotene solutions on the blood glycolysis of normal and of cancerous persons will be discussed in a later section. It was also reported that the addition of lactic acid caused a considerable depression of the glycolysis of blood derived

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[163] A. Caló: Boll. Acad. med. Roma *60*: 80; 93 (1934).

[164] A. Caló: Boll. Acad. med. Roma *61*: 152 (1935).

[165] A. Caló: Acta Cancrol. *1*: 437 (1935).

[166] G. H. Hitchings, R. H. Oster & W. T. Salter: Biochem. J. *32*: 1389 (1938).

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[167] Z. Dische & D. Laszlo: Biochem. Ztschr. *175*: 412 (1926).

[168] Y. Okamoto: Proc. Imp. Acad. (Tokyo) *3*: 184 (1927).

[169] I. Neumann: Vopr. Onkol. *8*: 90 (1935); abstr. Acta Cancrol. *2*: Ref. 24 (1936).

[170] K. Tadenuma & J. Homma: Deutsche med. Wchnschr. *50*: 1051 (1924).

[171] M. Ascoli & R. Indovina: Klin. Wchnschr. *13*: 956 (1934).

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172. M. Ascoli: Compt. rend. Soc. de biol. *119*: 902 (1935).
173. G. d'Alessandro: Ztschr. f. Krebsforsch. *43*: 452 (1936).
174. W. Fabisch: Biochim. e terap. sper. *22*: 206 (1935).
175. R. Indovina & V. Bevilotti: Biochim. e terap. sper. *23*: 406 (1936).
176. P. Mauriac: Compt. rend. Soc. de biol. *105*: 28 (1930).
177. P. Meyer-Heck: Ztschr. f. Krebsforsch. *49*: 142 (1939).
178. E. J. Sterkin, K. A. Iliada & M. S. Kosarnovskaja: abstr. Am. J. Cancer *31*: 123 (1937).
179. H. Werner & A. Kleinzeller: Am. J. Cancer *39*: 100 (1940).

from normal persons; this effect was less pronounced in blood of normal persons of greater age, and was least noticeable in cancer patients.[180]

One group of workers attempted to prove that the alteration of the metabolism, manifested chiefly as a high aerobic glycolysis, is not only specific for neoplasia, but even precedes the actual development of malignant tumors.[181] In support of this theory findings were presented which showed that the metabolism of the tumor-free organs of animals bearing tumors closely resembles the metabolism of tumors. Furthermore it was pointed out that prolonged administration of tar, of indole or of arsenic resulted in an increase of aerobic and anaerobic glycolysis and in a depression of the respiration in the organs of the animals treated, prior to the morphological appearance of malignancy. The authors thus felt justified in assuming that the metabolic alterations constituted an important factor in the predisposition to neoplasia. Confirmation has been furnished for the changed metabolic qualities of organs—which are free of tumors—of mice with spontaneous tumors. The liver, kidney and muscle of these animals exhibited an increased glycolysis and a lowered respiration.[182, 183] However, it appears very doubtful whether the theory mentioned above[181] is acceptable on the basis of the evidence available. It was reported that mice treated with benzpyrene exhibited changes of their oxidative metabolism only when tumors had already developed, but not in the preceding "preparatory" phase and, accordingly, the alterations of the tumor metabolism were regarded as a secondary phenomenon.[184] Furthermore, objections against associating the predisposition to cancer with metabolic disturbances have been raised on the basis of the following observations. Tissues adjacent to human neoplasms were found to show a normal metabolism whereas alterations of the metabolism similar to those encountered in tumors were found in various nonmalignant tissues undergoing degenerative processes.[185] In this connection reference must be made to the opinion that the metabolic properties described for neoplastic tissues are merely expressions of an impaired cell vitality, and therefore are not characteristic for these tissues.[186] More recently the specific nature of the tumor metabolism has also been doubted by other authors,[187, 188] while some investigators were inclined to retain their belief in Warburg's original conception of directly connecting the changed carbohydrate metabolism with the neoplastic process.[118, 189] There is need for renewed attempts at an analysis of these problems, some of which will be described in the following.

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[180] W. Pampe: abstr. Ztschr. f. Krebsforsch. *45*: Ref. 152 (1937).

[181] B. Fischer-Wasels: Gastherapie bösartiger Geschwülste, Frankfurt a. M. 1929.

[182] A. J. Sinay: abstr. Am. J. Cancer *31*: 114 (1937).

[183] I. M. Neumann: Bull. Biol. & Méd. Exper. URSS *2*: 247 (1936).

[184] L. Th. Larionow: Cancer Research *1*: 860 (1941).

Aerobic glycolysis of normal tissues. It is obvious that the results of these investigations may become of great importance for ascribing or denying a specific nature to the carbohydrate metabolism of malignant tumors. Warburg[88] first contributed to this field by calling attention to the fact that the gray matter of the brain and the retina exhibit a high anaerobic and aerobic glycolysis in a manner similar to that of cancerous tissue, and even surpassing it quantitatively. He originally believed that this phenomenon may be due to injuries sustained by these very sensitive tissues in the course of their experimental preparation. Later, however, he appeared to favor a different idea, namely, that the same source of biological energy may serve different purposes, in malignant tumors and in the central nervous system.[190] Another author [191] believed that the processes leading to the accumulation of lactic acid differ principally in the cases of tumors or of brain tissue. In the latter case, he assumed that a real increase of lactic acid formation took place, while in tumors the re-synthesis of lactic acid to hexoses was assumed to be disturbed (Pasteur-Meyerhof effect, see p. 515). In the case of some tissues which were reported to show aerobic glycolysis, these findings have been proved to be experimental artifacts. This applies for white blood cells which glycolyzed aerobically when suspended in a saline solution,[192, 193] whereas a normal metabolism was found in leucocytes suspended in serum derived from the same species.[194, 195] Similarly, the aerobic glycolysis of placenta disappeared when serum was used as the medium.[195] It is interesting that one type of malignant tumors, namely the chorioepithelioma, also behaved in the same manner as placenta;[195] this may be connected with other biological peculiarities of this neoplasm (frequent spontaneous disappearance of metastases following radical removal of the primary tumor). The fact that regenerating tissues also showed an increased aerobic glycolysis and a decreased respiration was interpreted as indicating a connection of these metabolic alterations with growth processes in general.[88, 196]

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[185] B. Walthard: Ztschr. f. Krebsforsch. *40*: 447 (1934).

[186] H. Druckrey: Arch. f. exper. Path. u. Pharmakol. *180*: 231 (1936); Deutsche med. Wchnschr. *62*: 717 (1936); Tumori n.s. *10*: 345 (1936).

[187] E. Boyland: Nature, London *145*: 246; 513 (1940).

[188] I. Berenblum, E. Chain & N. G. Heatley: Nature, London *145*: 778 (1940).

[189] F. Dickens & H. Weil-Malherbe: Nature, London *145*; 512; 779 (1940).

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[190] O. Warburg: Biochem, Ztschr. *204*: 482 (1929); *228*: 257 (1930).

[191] R. Bierich & A, Rosenbohm: Ztschr. f. physiol. Chem. *214*: 271 (1932).

[192] W. Fleischmann: Biochem. Ztschr: *184*: 385 (1927).

[193] A. Bakker: Klin. Wchnschr. *6*: 252 (1927).

[194] O. Warburg: Biochem. Ztschr. *187*: 480 (1927).

[195] A. Fujita: Biochem. Ztschr. *197*: 175 (1928).

[196] F. Pentimalli: Ztschr. f. Krebsforsch. *25*: 347 (1927).

A similar factor—an active growth capacity—was believed to be responsible for a remarkable glycolytic activity of fibroblasts cultivated in vitro.[197-199] A different opinion as to the significance of glycolytic activity was formed when it was found that roentgen irradiations of tissues resulted in an increase of the aerobic glycolysis when small doses were applied, whereas larger roentgen doses depressed all metabolic processes. These phenomena were interpreted as indicating that cell injury of a sublethal degree resulting, for instance, from suitable doses of irradiation may be the real cause of the occurrence of aerobic glycolysis. Hence this metabolic quality of neoplastic tissue might be regarded as a secondary phenomenon, i.e., dependent on cell damage.[200] On the other hand, a number of instances have become known of normal tissues which exhibit aerobic glycolysis, without it being possible to explain this quality by the existence of cell injuries. A high aerobic glycolysis was established for renal medulla [201] and, in contrast to earlier reports,[202] this glycolysis did not disappear when the test was performed with tissue suspended in serum derived from the same species.[201] Likewise, jejunal mucous membrane was found to form large amounts of lactic acid aerobically,[203] and determinations of aerobic and anaerobic glycolysis of normal rabbit skin and of Shope rabbit papilloma led to almost identical results which were also similar to values cited for squamous cell carcinomas.[204] It is accordingly understandable that serious doubts arose as to

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197. H. Laser: Biochem. Ztschr. *264*: 72 (1933); Klin. Wchnschr. *12*: 1933 (1933).
198. R. Meier: Biochem. Ztschr. *231*: 253 (1931).
199. A. Caló: Boll. Acad. med. Roma *60*: 97 (1934).
200. K. Inouye: Klin. Wchnschr. *15*: 613 (1936).
201. F. Dickens & H. Weil-Malherbe: Biochem. J. *30*: 659 (1936).
202. P. György, W. Keller & Th. Brehme: Biochem. Ztschr. *200*: 356 (1928).
203. F. Dickens & H. Weil-Malherbe: Biochem. J. *35*: 7 (1941).
204. I. Berenblum, E. Chain & N. G. Heatley: Am. J. Cancer *38*: 367 (1940).

the specific nature of the tumor metabolism described in the preceding parts. However, these questions must be regarded as still being undecided, and particular attention must be devoted to an approach as yet insufficiently investigated, i.e., to the metabolism of tissues undergoing malignant transformation during exposure to carcinogenic stimuli.

Experimental modification of carbohydrate metabolism. This subject has stimulated a large number of studies which were undertaken for two reasons. First they were expected to establish characteristic differences which may exist between normal and neoplastic tissues, as manifested in a different metabolic response to the same agent, and secondly, endeavors were made to modify the tumor metabolism in a manner which might be accompanied by an effect detrimental to tumor growth, this latter approach serving as a basis for numerous measures of chemotherapy discussed in other parts of this book.

Mechanical injuries as, for instance, grinding and mashing of tumors have previously been mentioned as being followed by a more or less complete loss of the glycolytic activity of neoplastic tissue[127, 128, 161, 205] but, at the same time, it is recalled that under special experimental conditions cell-free tumor extracts retain their glycolytic activity.[cf. 181] Inorganic compounds of biological importance, viz., potassium and calcium salts, were recognized as being requisite for the glycolysis of neoplastic tissue, since the absence of the ions depressed the rate of production of lactic acid.[206] It must be stressed that the absence of calcium and of potassium also lowered the glycolytic activity of other tissues which proliferate strongly, such as spleen, intestinal mucous membranes and lymph nodes, but it did not affect "stationary" tissues, such as the liver and kidney. A more detailed analysis

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205. B. R. G. Russel & W. E. Gye: Brit. J. Exper. Path. *1*: 175 (1920).

206. A. Lasnitzki & O. Rosenthal: Biochem. Ztschr. *207*: 120 (1929); *262*: 203 (1933).

showed that potassium is capable of directly activating the glycolytic process, whereas the effect of calcium is principally that of tightening the cell membranes, thus preventing a diffusion of potassium from the cells into the surrounding medium.[207, 208] Magnesium was found to react in a manner similar to calcium.[209] Other authors, however, failed to observe characteristic effects from the action of inorganic salts on the metabolism of tumor tissues,[210] and it is accordingly possible that these influences depend on special experimental conditions.

Sodium fluoride is generally known as an antiglycolytic substance and, therefore, it is not surprising that this compound impedes the formation of lactic acid in tumors.[211, 212] However, the glycolysis of neoplasms was found more sensitive to sodium fluoride than the glycolysis of normal tissues. It was also reported that the respiration of tumors was increased by sodium fluoride, whereas the respiration of normal tissue was scarcely affected.[212] Organic compounds with antiglycolytic properties are represented by halogen-substituted aliphatic acids, particularly monoiodoacetic acid, which compounds were studied extensively by Lundsgaard.[160] A complete suppression of the glycolysis was achieved in neoplastic tissue cultures by means of this substance. These tissues were found to be more sensitive than normal tissues, and reinoculation of neoplastic tissue treated with iodoacetic acid proved a complete loss of vitality.[213] Therapeutic experiments carried out with these substances were discussed in chapter

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207. A. Lasnitzki: Biochem. Ztschr. *264*: 285 (1933); 1st Internat. Cancer Congress, Madrid *2*: 16 (1933).
208. A. Lasnitzki & O. Rosenthal: Biochem. Ztschr. *281*: 395 (1935).
209. A. Lasnitzki: Biochem. Ztschr. *285*: 101 (1936).
210. F. Dickens & G. D. Greville: Biochem. J. *29*: 1468 (1935).
211. W. Ewig: Klin. Wchnschr. *8*: 759; 839 (1929).
212. C. Sellei & J. Jány: Biochem. Ztschr. *239*: 94 (1931).
213. A. A. Krontowski, M. K. Jazimirska-Krontowska & H. P. Ssawitzka: Ztschr. f. Krebsforsch. *37*: 457 (1932).

II, but in this connection emphasis may be placed on the fact that the suppression of glycolysis which follows the addition of sodium fluoride or of halogen fatty acids is known primarily to involve an inhibition of phosphorylation, as previously mentioned. In satisfactory agreement with the assumption of this mechanism, it was reported that the production of lactic acid occurring in tumor extracts, to which hexose phosphates had been added,[130] was not impeded by sodium fluoride or by monoiodoacetic acid.[214] It is obvious that under these experimental conditions the inhibition of phosphorylation cannot influence the glycolytic process. A different form of action probably underlies the effects of a number of chlorine compounds (monochloracetal, α-chlorhydrin, α-chlordiethylketone and mono-chloracetone) which, in a recent study, were found to inhibit the cell glycolysis in vitro in a reversible manner. They impeded the rate of tumor induction in mice painted with benzpyrene or dibenzanthracene to approximately the same degree as they inhibited the glycolysis.[215]

Interesting effects have been observed in vitro from the addition of ferric cyanide. This substance selectively suppressed the aerobic glycolysis of tumor cells. In the presence of ferric cyanide and of oxygen the tumor glycolysis dropped to approximately fifteen per cent, but returned to the original value under anaerobic conditions. These alternating effects could be repeated several times.[216, 217] Therapeutic experiments with ferric cyanide were suggested, but up to now none has been recorded. On the other hand, the action exerted by glyceric aldehyde extends, principally, to the anaerobic glycolysis. Earlier reports indicated that sufficiently large concentrations of this compound completely suppressed the anaerobic glycolysis of Jensen rat sarcomas, while the respiration was not affected.[218] It seemed also of interest that the related compounds dioxyacetone and acetol failed to interfere with the glycolysis, but markedly inhibited the respiration.[219] In later work, the inhibition of tumor glycolysis caused by glyceric aldehyde was also found to extend to nonmalignant tissues, and to be present only when glucose or glycogen formed the carbohydrate substrate but not with hexose phosphates.[220] Continued development led to the following results: glyceric aldehyde inhibited the anaerobic glycolysis of all "glycolyzing" tissues, such as tumors, brain, testis and embryo, whereas the compound stimulated the glycolysis in liver and kidney. The production of lactic acid from methylglyoxal by tumor tissue was not affected by glyceric aldehyde.[221]

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[214] F. H. Scharles, M. D. Baker & W. T. Salter: Biochem. J. *29*: 1927 (1935).

[215] H. G. Crabtree: J. Path. & Bact. *51*: 303 (1940).

[216] B. Mendel: Am. J. Cancer *30*: 549 (1937).

[217] B. Mendel & E. Strelitz: Nature, London *140*: 771 (1937).

In a number of studies devoted to the analysis of tumor glycolysis, a substance not hitherto thought to be connected with the carbohydrate metabolism, viz., the carotene, was employed. This work was motivated, in part, by several earlier investigations which had clearly indicated that carotene, by virtue of its great affinity for oxygen, may influence various biological oxidation processes.[222-226] The first data regarding the effect of carotene on glycolytic processes concerned the blood glycolysis, and may be summarized as follows. The addition of suitable aqueous emulsions of carotene to red blood cells in general caused a marked increase of the glycolysis, but as a notable exception to this rule, erythrocytes of tumor patients failed to react in the same manner. That is, in the majority of the latter cases, carotene either did not affect the blood glycolysis at all, or even depress it.[227] The use of the appearance of this phenomenon, which later was confirmed in its essentials,[175] was tentatively proposed for diagnostic purposes (cf. chapter X). A closer study was made possible by the observation that carotene solutions also markedly increased the formation of lactic acid in Lohmann's muscle extracts.[228] This substrate permitted the reasonably accurate determination of the phase of glycolysis affected by the carotene. Instead of the active muscle extract, a substrate was used, which had been deprived of its coferment by dialysis and therefore did not glycolyze. A re-activation of this system was then achieved by means of the addition either of boiled muscle extracts, boiled yeast extract, or of boiled tumor extracts. Significantly, these new systems reacted differently in the presence of carotene. Whereas the glycolysis of substrates activated by muscle or yeast extracts was considerably increased after the addition of carotene, substrates supplemented by tumor extracts exhibited a depressed glycolysis in the presence of carotene.[229] The stimulating effect of carotene on glycolysis was tentatively explained as depending on the property of binding oxygen, which is inherent in carotene. In this manner, it was assumed, carotene may create a relative anaerobiosis, that is, conditions favorable for fermentation. This view appeared supported by the following experimental results. Blood in which an anaerobiosis had been produced by means of cyanide did not exhibit a further increase of glycolysis after the addition of carotene. On the other hand, red blood cells of birds, which possess nuclei and a strong respiration, do not glycolyze normally but, after the addition of carotene, glycolysis takes place.[227] Furthermore, it was shown that the ability of carotene to bind oxygen is not confined to molecular oxygen, but extends also to oxidative systems ("Zwischenkatalysatoren"). Thus, the complete suppression of the glycolysis which can be caused by quinone [230] was nullified by a simultaneous addition of carotene.[231] This finding made it very probable that carotene has the property of paralyzing the effect of oxidative systems, such as quinone, and thus of inhibiting the Pasteur-Meyerhof effect. Since it was found that a glycolytic system, which was activated by boiled tumor extracts, failed to be influenced by carotene in the same manner as normal glycolytic systems, it may be concluded that the tumor substrate is characterized by a deficiency of oxidative systems sensitive to carotene. Further experiments showed the effect of carotene also to be absent in those glycolytic systems which had been activated by mixtures of boiled muscle and boiled tumor extracts.[231] This may indicate that the tumor extract is not only deficient in certain oxidative systems, but is also endowed with some positive qualities enabling it to modify the behavior of "normal" glycolytic systems. In this connection it is possible to assume a reducing property in the tumor extract. Actually, boiled tumor extracts were found to destroy the effect of the addition of quinone, which substance, as mentioned above, stimulates the Pasteur-Meyerhof effect and thus inhibits the glycolytic process. Hence tumor extracts may be assumed to contain factors capable of interfering with oxidative systems. These reducing factors may play an important part in tumor metabolism, and may be responsible for the high aerobic glycolysis of neoplasms. In other words, they may represent the cause of the lesser effectiveness of respiration (oxygen consumption) which Warburg assumed to exist in tumors. Specifically, a disturbance of some catalysts of oxidation may result in the phenomenon that neoplasms, although consuming normal quantities of oxygen, utilize these amounts in a faulty manner, inasmuch as the oxygen would be intercepted by reducing systems before it could act.

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[218] B. Mendel: Klin. Wchnschr. *8*: 169 (1929).

[219] B. Mendel: Klin. Wchnschr. *9*: 118 (1930).

[220] E. Adler, F. Calvet & G. Günther: Ztschr. f. physiol. Chem. *249*: 40 (1937).

[221] Z. Baker: Biochem. J. *32*: 332 (1938).

[222] Arnaud: Compt. rend. Acad. d. sc. *100*: 751 (1885); *102*: 1129; 1319 (1887); *109*: 911 (1889).

[223] R. Willstätter & W. Mieg: Ann. *355*: 1 (1907).

[224] B. R. Monoghan & O. F. Schmitt: J. Biol. Chem. *96*: 387 (1932).

[225] A. Fodor & R. Schoenfeld: Biochem. Ztschr. *233*: 243 (1931).

[226] W. Franke: Ztschr. f. physiol. Chem. *212*: 234 (1932).

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[227] C. Wetzler-Ligeti & R. Willheim: Biochem. Ztschr. *254*: 364 (1932); Acta Cancrol. *1*: 289 (1935).

[228] C. Frisch & R. Willheim: Biochem. Ztschr. *272*: 332 (1934).

[229] C. Frisch & R. Willheim: Biochem. Ztschr. *272*: 337 (1934).

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[230] F. Lipmann: Biochem. Ztschr. *265*: 133 (1933).

[231] C. Frisch & R. Willheim: Biochem. Ztschr. *277*: 148 (1935).

The preceding remarks obviously imply a disturbance of the Pasteur-Meyerhof reaction in malignant tumors, although the existence of such a change was not definitely proved and represents a rather controversial problem. Pasteur was the first to point out clearly that fermentation is inhibited or suppressed in the presence of oxygen. An analogous effect of oxygen was found to extend to glycolytic processes, phenomena studied extensively by Meyerhof, whose name, therefore, frequently is attached to this effect. Warburg, in his later work, was inclined to regard this effect as being disturbed in neoplastic tissues. That is, he attributed the high aerobic glycolysis of tumors, not merely to a diminished extent (quantity) of the oxygen consumption, but he also connected the glycolysis with a reduced effectiveness (quality) of the oxygen consumed. Conclusions of a similar nature were derived by Dickens[281] from his experimental work, in which he studied the influence of various substances on the Pasteur effect in normal and in tumor tissues. Yet, on the other hand, Burk[92] strongly opposed the contention that the Pasteur effect is less active or disturbed in neoplastic tissue, and he supported his viewpoint by the results of experimental work and by a critical analysis of the results obtained by other authors. According to Burk, tumors are chiefly characterized by a high aerobic glycolysis and a diminished oxygen consumption, a problem which will be taken up in detail in the section on respiration. Notwithstanding these objections, the question of the Pasteur effect in tumors cannot be considered as closed, and a new approach to its elaboration may have been opened by recent studies which showed that the Pasteur effect is linked with a specific enzyme.[233, 233a]

Reference may be made once more to the hypothesis which regarded reducing substances present in tumors as responsible for an "oxygen shunt" before the respiration can exert its effect on the carbohydrate metabolism. Bierich[231] assumed that cysteine might possess a special significance as a reducing compound and as activating the glycolysis. Under special experimental conditions, the sulfhydryl compound glutathione has been found to increase the glycolysis of tumor substrates,[234] but another worker observed a general ineffectiveness of glutathione regarding the glycolysis of normal and of neoplastic tissues, and he stated that this compound did not influence the Pasteur effect.[235]

Ascoli and his school[236, 237] (cf. chapter X), ascribed to unsaturated fatty acids an important part in the mechanism of oxygen interception and in the subsequent stimulation of glycolysis in tumor tissue. Actually, an increased glycolysis of red blood cells was seen after the addition of unsaturated fatty acids,[238] although another investigator arrived at somewhat divergent results. The glycolysis of normal erythrocytes was increased by seventy-five per cent after the addition of methyl alcoholic extracts of Jensen rat sarcomas, of the

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232. F. Dickens: Biochem. J. *33*: 2017 (1939).
233. K. G. Stern & J. L. Melnick: J. Biol. Chem. *139*: 301 (1939).
233a. K. G. Stern, J. L. Melnick & D. DuBois: Science *91*: 436 (1940).
234. C. A. Mawson: Biochem. J. *30*: 1592 (1936).
235. Z. Baker: Biochem. J. *31*: 980 (1937).
236. M. Ascoli: Klin. Wchnschr. *14*: 1593 (1935).
237. R. Indovina & W. Fabisch: Ztschr. f. Krebsforsch. *42*: 63 (1935).
238. G. d'Alessandro & S. Petrucci: Biochem. Ztschr. *285*: 72 (1936).

serum or of the red blood cells of tumor patients, whereas analogous extracts derived from normal substrates caused a stimulation of only twenty per cent. The active factor, however, of these extracts was believed to be lipids instead of fatty acids.[239]

A different form of action appears to be involved in the stimulation of glycolysis in Jensen rat sarcomas which, it was observed, resulted from the addition of pyruvic acid, of methylene blue, or of potassium ferricyanide.[240, 241] These substances are oxidants which may be reduced by the tissue and thus act as acceptors of hydrogen during glycolytic processes. These compounds most probably participate in the oxidation of trioses derived from the hexose cleavage. The glycolysis of tumor slices was found to be stimulated by dinitrophenol derivatives, such as dinitrophenol-o-cresol.[242] Phenylhydrazine did not affect the anaerobic glycolysis but increased the aerobic glycolysis almost to the level of the anaerobic glycolysis.[243] It seems of interest that the addition of the carcinogen 1,2,5,6-dibenzanthracene to liver slices increased the glycolysis in vitro, only when the hepatic tissue was derived from animals previously treated with this substance in vivo; the liver tissue of non-treated animals failed to be affected by the carcinogen.[244] Another investigator provisionally reported that the aerobic glycolysis of embryonic chick heart was increased after the addition of benzpyrene; this effect, however, was detectable only after twenty-four hours of interaction of the carcinogen and the substrate.[245] The evidence thus far presented as to the direct influence of

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239. W. Fabisch: Am. J. Cancer *28*: 764 (1936).
240. O. Rosenthal: Ztschr. f. Krebsforsch. *38*: 216 (1932).
241. F. Dickens: Nature, London *134*: 382 (1934).
242. E. C. Dodds & G. D. Greville: Lancet *1*: 398 (1934).
243. F. Dickens: Biochem. J. *28*: 537 (1934).
244. G. Boström: Acta radiol. *16*: 224 (1935); abstr. Am. J. Cancer *25*: 169 (1935).
245. G. F. de Gaetani: Acta Cancrol. *2*: 355 (1936).

carcinogens on metabolism is obviously too scant to permit any definite conclusions.

Protein metabolites have been assumed to be connected with the tumor glycolysis; at least, the glycolysis of rat sarcomas was reported to be impeded in vitro by alanine, leucine and tryptophane, whereas cysteine stimulated the production of lactic acid.[246] The search for chemical agents which suppress the tumor glycolysis has been motivated in part by the idea that such substances might prove useful for the treatment of cancer (cf. chapter II). In contrast to the glycolysis of muscle, the tumor glycolysis was found to be impeded by anesthetics,[87] but a practical utilization of this effect is hardly feasible. An antiglycolytic quality was observed of several aniline dyes,[247] most of which, however, are very toxic. It is, nevertheless, worth noting that a number of azo dyes which do not act as protoplasm poisons were shown to suppress the tumor glycolysis and even, in some instances, were successfully used for causing regressions of transplanted mouse tumors.[248, 249] Reference must be made also to an inhibition of tumor glycolysis which can be achieved by a number of organic compounds (sodium salts of pyrocatechin disulfonic acid, glycine, hexose diphosphate, etc.), all of which are known to form complex salts with heavy metals. Hence, these substances may be supposed to interfere with glycolysis by means of an inactivation of heavy metal catalysts.[250] It is probable that this relates in particular to copper catalysts, and the substances named were also shown to impede the glycolysis in vivo.[251] On the other hand, the glycolysis of malignant tumors was found to be more sensitive to metallic poisons than the glycolysis of normal tissues.[252] An almost complete elimination of the aerobic glycolysis of tumors was achieved with thionine.[241] However, 2,6-dichlorophenol indophenol was incapable of impeding the tumor glycolysis, in contrast to its marked effect on muscle glycolysis.[253]

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[246] S. Miyao: Tr. Jap. path. soc. *22*: 992 (1932).
[247] M. Yabusoe: Biochem. Ztschr. *168*: 227 (1926).
[248] L. Karczag: Biochem. Ztschr. *230*: 411 (1930).
[249] L. Karczag & C. Sellei: Biochem. Ztschr. *230*: 420 (1930).
[250] G. Hecht & F. Eichholtz: Biochem. Ztschr. *206*: 282 (1929).
[251] F. Eichholtz & K. Baumgart: Arch. f. exper. Path. u. Pharmakol. *178*: 161 (1935).

Brief mention is made of a few investigations on the influence of hormones on the tumor glycolysis. Anterior pituitary extracts were reported to have reduced the glycolysis of uterine carcinomas in vitro by thirty-six per cent.[254] However, the metabolism of tumors of hypophysectomized rats was not found to differ from that of tumors growing in control animals.[255] Sarcoma-bearing rats were injected with various hormones and, after sacrifice of the animals, the lactic acid production was determined in the tumors. In these experiments, insulin and extracts of the posterior pituitary were observed to lower the tumor glycolysis, whereas the administration of adrenalin, estrogens, gonadotropic hormones and of preparations of parathyroid or thyroid was ineffective.[256] Another worker claimed that the effect of insulin depended on its dosage; small amounts added to tumor slices lowered the glycolysis and increased the respiration, whereas large doses exerted the opposite effects.[257]

Effects of roentgen irradiation. It was of particular interest to ascertain changes of the tumor metabolism which may follow roentgen or radium irradiation, in view of their great therapeutic effectiveness. It was reported that tumor tissue, following irradiation in vivo, in vitro showed a de-

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252. M. Jowett & J. Brooks: Biochem. J. *22*: 720 (1928).
253. K. A. C. Elliott: Nature, London *134*: 254 (1934).
254. L. Kriesch & K. Vietorisz: Zentralbl. f. Gynäk. *58*: 2370 (1934).
255. C. C. Franseen & C. McTiernan: Am. J. Cancer *26*: 106 (1936).
256. W. Heinrichs: Thesis, Königsberg i. Pr., 1933; abstr. Ztschr. f. Krebsforsch. *41*: Ref. 21 (1935).
257. S. F. Gomes da Costa: Bol. inst. para el estud. y trat. d. cáncer *12*: 619 (1935).

crease of the glycolytic activity, apparently in proportion to the therapeutic effect of the radiation.[258] On the other hand, neoplastic tissue cultures treated with roentgen doses which completely suppressed their growth were found unchanged regarding their metabolic properties[259] and, likewise, the radium doses required to inhibit the glycolysis of cancer tissue cultures were reported to be considerably higher than the doses required to impede the tissue growth.[260] These phenomena have been extensively investigated during recent years, and the following results, which contribute to a clarification of this problem, were obtained.[261–263] Radiation doses which suppressed the growth in vitro failed to affect glycolysis and respiration; much larger doses were required for reducing these metabolic processes. This was found both for normal kidney tissue of mice and in mouse sarcoma 180, although in the latter a stronger inhibition of the glycolysis could be achieved than in normal tissue with the same amount of radiation. Precise analyses of the effects of variation of the irradiation doses, and of the time of exposure, were presented, and special significance may be ascribed to comparisons of the radiation effect in vitro and in vivo. In the vitro experiments, much higher roentgen quantities were required for suppressing growth and metabolism than in experiments with living animals, and this appears to be in satisfactory agreement with other observations which suggested that not only local reactions, but systemic factors also, contribute to the effect of tumor irradiation in vivo (cf. chapter VIII). An

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258. W. Haarmann: Biochem. Ztschr. *280*: 173 (1935).

259. A. A. Krontovsky & E. G. Lebensohn: Vopr. Onkol. *6*: 86 (1934); abstr. Ztschr. f. Krebsforsch. *42*: Ref. 41 (1935).

260. M. J. Kolomijetz: Arch. f. exper. Zellforsch. *16*: 260 (1934).

261. A. Goldfeder: Radiology *31*: 73 (1938).

262. A. Goldfeder & J. L. Fershing: Radiology *31*: 81 (1938).

263. A. Goldfeder: Am. J. Cancer *36*: 603 (1939); Compt. rend. Soc. de biol. *130*: 571 (1939).

interesting finding was reported, according to which roentgen irradiation of neoplastic tissue caused a much more pronounced depression of glycolysis, when applied at low temperatures, as compared with the effect obtained after irradiation at body temperatures. Normal tissue did not react differently to radiations administered at low, or at body temperatures.[264]

Attempts were made to influence the tumor glycolysis by means of biological substrates, the chemical nature of which is not yet defined. Thus, spleen extracts were reported to stimulate the respiration and to lower the glycolysis of mouse tumors [265] and, in satisfactory agreement with this observation, another author found an increased glycolysis of tumors growing in splenectomized animals.[266] Additional relations between the spleen and carbohydrate metabolism will be considered in chapter VIII.

Announcements were made of the isolation from yeast and normal mouse tissues of factors which stimulate respiration. These agents were reported to depress the anaerobic and aerobic glycolysis in normal as well as in neoplastic tissues.[267] So far no confirmation of these phenomena has been made known. That a rather cautious evaluation of the aforementioned claims is necessary is suggested by a study, which showed that crude alcoholic extracts of baker's yeast are capable of strongly stimulating the respiration of normal liver and of the skin of mice, but they were ineffective in a spindle cell mouse sarcoma.[268] On the other hand, it was disclosed that during pregnancy, and also in some instances of neoplasia, a factor which inhibited the fermentation of yeast was found in the blood.[269] A later study confirmed the stronger "antizymatic" action of the blood of tumor patients and sterol-like compounds were supposed to be responsible for this inhibition.[270]

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[264] H. G. Crabtree: Biochem. J. *28*: 2334 (1935).

[265] W. Büngeler: Frankf. Ztschr. f. Path. *39*: 314 (1930); *43*: 409 (1932).

[266] H. Matsuoka: Jap. J. Obst. & Gynec. *17*: 25 (1934).

[267] M. P. Schroeder & E. S. Cook: Studies of the Institutum Divi Thomae *2*: 247 (1939); abstr. Am. J. Cancer *40*: 272 (1940).

[268] M. J. Carroll: Nature, London *143*: 684 (1939).

The investigations thus far described were based, implicitly, on the assumption that a suppression of the tumor glycolysis is desirable because this may simultaneously cause a damaged vitality and a lowered rate of growth of the neoplasms. But at least one author [271] proceeded from just the opposite premise, i.e., he believed it feasible to kill neoplastic tissue by means of an excessive stimulation of its production of lactic acid. This was suggested to him by his observing that tumor slices lose their glycolytic activity in vitro with the accumulation of lactic acid; furthermore, higher lactic acid concentrations and a lower glycolytic activity were found in spontaneous necroses of tumors. The method of stimulating glycolysis consisted of injections of large quantities of glucose and of hypophysectomy of the tumor-bearing animals; the latter procedure was thought to increase the formation of lactic acid as a consequence of a depression of the oxygen consumption (cf. chapter VII). This treatment was reported to yield favorable results in animal experiments, but no additional confirmation or clinical applications of similar experiments have been recorded.

It was previously shown that the attempts to connect the etiology of malignant tumors with metabolic alterations met with objections because several observations tended to discredit the specific nature of the metabolic disturbances occurring in neoplastic tissue. Similarly, grave doubts arose as to the rationality of therapeutic measures which were motivated by the idea that interferences with the tumor metabolism will result in curative effects. These doubts were strengthened by several findings which demonstrated that the glycolytic activity of tumor tissue is independent of its growth rate.[251, 272-276] Thus tissue cultures of Jensen rat sarcoma were investigated in which the glycolysis was lowered by forty to seventy per cent, nevertheless, the growth of these cells was not changed.[277] Very illuminating results were revealed in a study in which the metabolism of tumors (mouse sarcoma 180) with different growth rates was compared. These tumors ranged from "benign" ones, growing very slowly in "immunized" animals, to very malignant ones with a rapid growth rate. However, no direct relation could be established between the growth rates and the production of lactic acid in the tumors. The oxygen consumption was found to be lower in the "benign" tumors.[278] Similarly, other workers were able to depress the intensity of growth of animal tumors by means of certain organic sulfur compounds or by means of dibenzanthracene. In spite of these effects, the glycolytic activity of these tumors did not differ appreciably from that of more rapidly growing tumors.[279] Notwithstanding these interesting observations, it would appear premature completely to deny the possibility that curative effects may be derived from procedures intended to restore the disturbed metabolism of tumor tissue to normal. Most likely the prac-

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[269] W. Funk: Fermentforsch. *16*: 1 (1938).
[270] H. Eufinger & Sprado: Monatschr. f. Geburth. *101*: 5 (1935).
[271] M. Reiss: 1st Internat. Cancer Congress, Madrid *2*: 410 (1933).

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272. F. Bischoff & L. C. Maxwell: J. Pharmacol. & Exper. Ther. *40*: 97 (1930).
273. E. McDonald: Science *74*: 55 (1931).
274. W. Keil: Arch. f. exper. Path. u. Pharmakol. *167*: 338 (1932).
275. F. Eichholtz, W. Keil & L. Kluge: Biochem. Ztschr. *260*: 139 (1933).
276. A. Reis & L. Kluge: Arch. f. exper. Path. u. Pharmakol. *181*: 281 (1936).
277. B. Snellman: Arch. f. exper. Zellforsch. *20*: 213 (1937).
278. J. Muus, F. N. Craig & W. T. Salter: Am. J. Cancer *37*: 553 (1939).
279. E. Boyland & M. E. Boyland: Biochem. J. *33*: 618 (1939).

tical success of this trend will greatly depend on theoretical factors, that is, on a more exact insight into the mechanisms of the metabolic alterations occurring during the very first stages of carcinogenesis.

4. *Mineral Metabolism.*

The discussion of metabolic changes in neoplasia which relate to minerals may be kept very brief since a great part of the findings on this subject have been described in chapter I. Moreover, the total number of studies devoted to these problems in recent times is small while reports of an older date are necessarily subject to cautious evaluation because of their methodological inadequacy or their obsolete theoretical background. To a certain extent, these limitations must be placed on the value of several studies which were performed in the last century, and which indicated a retention of sodium chloride as taking place in cancerous persons.[1, 280, 281] Some authors, moreover, failed to corroborate these findings[4, 282] and since, to the writers' knowledge, work on this problem has not been resumed in recent times, it awaits a final solution. In any case, it can hardly be expected that the excretion of sodium chloride in neoplasia is governed by factors other than those decisive in general physiology and pathology. Hence, deviations from the normal chloride metabolism, which may be observed in tumor patients, are most probably secondary effects dependent on the water metabolism, on the kidney function, and on dietary factors, etc. Similarly, no primary connection with the neoplastic process can be assumed to exist regarding an increased urinary excretion of phosphates by tumor hosts, as reported by some authors.[1, 282] This phenomenon probably represents a consequence of the

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280. G. Sticker & C. Hübner: Ztschr. f. klin. Med. *12*: 114 (1887).
281. W. von Moraczewski: Virchows Arch. f. Path. *139*: 385 (1895).
282. R. Laudenheimer: Ztschr. f. klin. Med. *21*: 513 (1892).

destruction of tissues rich in cell nuclei and, accordingly, rich in proteins containing phosphorus. It was also claimed that the urinary excretion of calcium is, in general, diminished in neoplasia. However, the formation of bone metastases was found to be accompanied by a sudden increase of the urinary calcium values.[283] It is needless to add that this is to be interpreted as being a consequence of osteolysis and, like the findings previously mentioned, these alterations are in no way specific for malignant diseases. A recent paper reported on the mineral metabolism of mice painted with benzpyrene; the urinary chloride excretion was found to decrease with the appearance of papillomas and, particularly, with the development of malignancy, whereas the excretion of phosphates and sulfates was increased during the same period.[284]

The introduction into the body of radioactive isotopes of various elements is a recent trend, rendered possible by the discoveries of atomic physics. As shown in chapter 1, investigations of this kind of neoplasia were performed with radioactive ("labelled") phosphorus, and led to interesting findings regarding a different rate of uptake and metabolism of this element in normal and in tumor tissues.[285-289] It remains to be seen whether significant findings of theoretical or practical value can be obtained from additional work employing radioactive isotopes of other elements as tracers of mineral metabolism in neoplasia.

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283. M. Weiss: Wien. klin. Wchnschr. *48*: 454 (1935).
284. A. I. Aivasian: Bull. Biol. et Méd. Exper. URSS *9*: 521 (1940).
285. H. B. Jones, I. L. Chaikoff & J. H. Lawrence: Am. J. Cancer *40*: 235; 243 (1940).
286. A. Marshak: Science *92*: 460 (1940).
287. L. W. Tuttle, L. A. Erf & J. H. Lawrence: J. Clin. Investigation *20*: 57 (1941).
288. J. M. Kenney: Surgery *9*: 327 (1941).
289. J. M. Kenney, L. D. Marinelli & H. Q. Woodard: Radiology *37*: 683 (1941).

5. *Respiration.*

The term respiration, for which the term oxygen consumption may more precisely be used, is employed for two groups of phenomena which, it is true, are closely linked with each other. One of them is the gas exchange taking place in the respiratory epithelium and in the capillaries of the lungs. The other summarily comprises the oxygen consumption necessitated by the combustion of oxidisable compounds in cellular and humoral media. Hence oxidative processes do not concern only one group of constituents of the body but, instead, practically all of them. For this reason, a discussion of oxygen consumption, and of the metabolic phenomena connected with it, may fittingly conclude this chapter.

Basal metabolic rate. The determination of the basal metabolism represents the established method of ascertaining the total oxygen consumption of an organism in vivo. By estimating the gas metabolism under standard conditions, values are obtained which can be compared with empirical standard values (basal metabolic rate), and thus pathological alterations may be recognized. However, as is generally known, no exact insight into the mechanism of intracellular oxidation can be expected to emerge from these findings which, so to speak, constitute a whole, the parts of which are unknown. Furthermore, a number of factors, particularly the function of the endocrines and the composition of the food, have a decisive influence on the basal metabolic rate. It is, accordingly, not surprising that the results of this test were neither uniform nor characteristic with persons with malignant tumors. It is true that several investigations reported an increased basal metabolic rate in tumor patients,[290-297] but the fact was

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[290] A. Heindl & R. Trauner: Mitt. a. d. Grenzgeb. d. inn. Med. u. Chir. *40*: 416 (1927).

[291] J. Mělka & J. Jakšy: abstr. Zentralbl. f. Chir. *55*: 2239 (1928).

[292] S. A. Holboll: Acta med. Scandinav. *72*: 475 (1929); abstr. Cancer Rev. *6*: 24 (1931).

stressed that the increase generally amounted to less than twenty per cent, which is below the border value accepted by most authors as indicating a definite deviation from the normal.[298] One investigator believed that the increase of the basal metabolic rate was proportional to the progress of the malignant disease,[295] but other workers failed to note any such relation.[290] The cachexia of tumor patients, which is an unspecific factor, was held to be responsible for the deviation from the normal [293] but, here again, contradictory views were expressed because high metabolic rates were also observed in cancerous persons with a satisfactory general condition; disturbances of the protein metabolism were suspected as being the cause of the increase of the basal metabolic rate.[292] Cancers of the gastro-intestinal tract were most frequently found to be accompanied by an increased basal metabolism [291–294] and some authors found that high values returned to normal after surgical removal of the neoplasms.[295, 296] On the other hand, contrary results were recorded by investigators, who observed reduced basal metabolic rates in tumor patients (minus four to minus thirty-five per cent).[291, 299, 300] These discrepancies may have been due, in part, to the selection of the cases, which explanation is the more plausible since in most of these studies only a small number of cases were tested. This view is supported by the results of a study dealing with fifty cancer patients—most of them cancers of

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293. L. Bazzicalupo: Gazz. internaz. med.-chir. *8*: 687 (1929); abstr. Cancer Rev. *5*: 726 (1930).

294. F. Strieck & H. B. Mulholland: Deutsches Arch. f. klin. Med. *162*: 51 (1929).

295. D. Biolato: Cancro *2*: 137 (1931).

296. V. Jura: Riv. di pat. sper. *4*: 35 (1935); abstr. Ztschr. f. Krebsforsch. *43*: Ref. 27 (1935).

297. E. Fischbach: München. med. Wchnschr. *84*: 1327 (1937).

298. A. Moratti: Tumori n.s. *8*: 1 (1934); Monit. Endocrinol. *2*: 235 (1934); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 103 (1935).

299. G. C. Peracchia: Tumori *14*: 326 (1928).

300. D. Donati: Tumori n.s. *4*: 126 (1930).

the female genital tract. The authors of this study concluded that, in a statistical sense, the average values of the basal metabolic rates in the tumor patients were higher than in persons free of malignancy, but they saw no basis for the assumption that neoplasia is characteristically associated with an increased basal metabolism.[301] Likewise, the few studies performed with tumor-bearing animals failed to indicate a primary influence of neoplasia on the basal metabolic rate. It was reported that in rabbits painted with tar a reduction of the basal metabolic rate occurred with the onset of precancerous lesions,[302] but it may be assumed that this effect was caused rather by the general toxic action of the tar than by its carcinogenic activity. Different types of neoplasms may affect the basal metabolism in different ways as was shown in the case of fowl tumors. Chickens with fibrosarcomas exhibited only slight increases of the basal metabolic rate, whereas animals bearing spontaneous lymphocytomas showed marked increases.[303]

Respiratory quotient. The determination of the respiratory quotient, i.e., of the ratio between carbon dioxide produced and oxygen consumed, permits a more definite evaluation of intracellular oxidative processes. When measuring the respiratory quotient of neoplastic tissue in vitro, values approximating unity may be expected to be found, in view of the fact that carbohydrates represent the main source of energy in this tissue. Significantly, however, precise studies performed with tumor slices in vitro yielded respiratory quotients definitely below unity.[304] It is conceivable that these findings indicate the occurrence of incomplete oxidation, thus resulting in a lesser amount of the finally produced carbon

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301. R. O. Bowman & H. C. Pitts: Am. J. Obst. & Gynec. *32*: 957 (1936).

302. A. Remond, M. Sendrail & Lassalle: Compt. rend. Soc. de biol. *93*: 979 (1925).

303. C. Olson & H. H. Dukes: Fol. hematol. *60*: 57 (1938).

304. F. Dickens & F. Šimer: Biochem. J. *24*: 1301 (1930); *25*: 973; 985 (1931).

dioxide. The lowering of the respiratory quotients was found especially pronounced in slowly growing tumors, a fact suggesting the possibility that, in addition to carbohydrates, other substrates, mainly fats, are also metabolized by these tumors.[305] Some authors failed to confirm the regular lowering of the respiratory quotient in neoplastic tissues,[306] or even reported that neoplastic tissue cultures showed markedly higher respiratory quotients than cultures of embryonic chick heart.[307] However, more recent studies, performed with a great number of chicken tumors, furnished new evidence for a reduction of the respiratory quotients in the great majority of the malignant tumors tested. This finding, together with the high aerobic glycolysis, was believed to be most characteristic for the metabolism of neoplasms.[113] It is noteworthy that the administration of roentgen irradiation in vitro caused a reduction of the respiratory quotient of Philadelphia rat sarcomas, whereas the administration of the same amount of radiation to rat kidneys had no measurable effect on the respiratory quotient of this tissue.[308] Furthermore, reference is made to some determinations in vivo of the respiratory quotient. Several authors disclosed that the administration of glucose to tumor patients caused a smaller rise of the respiratory quotient than was the case in noncancerous patients.[66, 309, 310] Animals painted with tar were reported to react in this manner even in the precancerous stage.[311] However, a definite interpretation of these findings of older date does not appear possible until new evidence is furnished by more extensive investigations of this problem.

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[305] B. R. G. Russel: Rep. Imp. Cancer Res. Fund, London, p. 1; 11 (1923).

[306] K. A. C. Elliott & Z. Baker: Biochem. J. *29*: 2433 (1935).

[307] A. H. Roffo & L. M. Correa: Bol. inst. med. exper. para el estud. y trat. d. cáncer *12*: 586 (1935); Bull. Assoc. franç. p. l'étude du cancer *25*: 95 (1936); Ztschr. f. Krebsforsch. *43*: 337 (1936).

[308] G. Bancroft & V. E. Kinsey: Biochem. J. *31*: 974 (1937).

[309] J. Geldrich & M. Heksch: Ztschr. f. klin. Med. *104*: 620 (1926).

[310] E. Joltrain, T. Révész & R. Wolff: Compt. rend. Soc. de biol. *98*: 589 (1928); Bull. Assoc. franç. p. l'étude du cancer *17*: 125 (1928).

[311] F. Silberstein, J. Freud & T. Révész: Wien klin. Wchnschr. *38*: 356 (1925).

Oxygen consumption by tumor substrates in vitro. A distinction may be made between those studies in which fresh tumor slices or neoplastic tissue cultures were used, and between those employing various substrates in which living tumor cells cannot be expected to be present. Investigations of the first type were usually combined with the metabolic studies on glycolysis, as described in preceding sections. In general, the oxygen consumption of cancerous tissue was found more or less reduced when compared with the respiration of normal tissues.[85, 87, 118] However, divergent opinions have been expressed regarding a very important aspect of respiration in neoplasms. This refers to the question as to whether in neoplasia only the extent of the respiration is changed, or whether its quality or effect may also be changed. Several authors answered this question in the affirmative, and thus considered the diminished effectiveness of the oxygen consumed by neoplastic tissue to be a very characteristic phenomenon (impaired Pasteur-Meyerhof effect). On the other hand, the validity of this view was strongly contested,[cf. 92] and additional work will probably be required before this problem can be definitely clarified.

The results derived from determinations of the oxygen consumption in substrates, prepared from tumors by grinding, extracting or by other measures which destroy cellular life, will largely depend upon the experimental conditions used; divergent results are accordingly to be expected. The oxygen consumption of tumor pulp was either not found appreciably to differ from that of normal substrates [312] or was even reported to exceed normal values.[313, 314] In general, the oxygen consumption of these substrates obviously depends

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312. B. R. G. Russel & W. H. Woglom: Brit. J. Exper. Path. *1*: 244 (1920).

313. N. Waterman & J. M. Dirken: Arch. neerl. physiol. *5*: 328 (1921).

314. A. Fleisch: Biochem. J. *18*: 294 (1924).

on two factors: the quantity of oxidisable substances, and the presence of specific enzymes facilitating the transfer of oxygen, or hydrogen. Investigations of these enzymatic oxidative systems (dehydrogenases) deserve great interest in connection with tumor metabolism, and findings discussed in the related section of chapter IV will be supplemented by additional data in the next section. The observation that filtrates of malignant tumors, in contrast to those of normal organs, did not exhibit any oxygen consumption[315] is significant, inasmuch as it may be indicative of the absence of certain oxygen acceptors, or of oxygen-transporting enzymes in the filtrates of neoplastic tissue. A similar interpretation may be applied to the following report. Boiled extracts of normal organs consumed oxygen both in the presence and in the absence of hexose monophosphates, while boiled tumor extracts failed to utilize oxygen in the absence of the hexose phosphate.[316] It was recently disclosed that a "thermostable powder," prepared from Flexner-Jobling rat carcinomas, which powder may be regarded as denatured protein, showed, in the presence of glutathione, a much lower oxygen consumption than did similar preparations derived from muscle. This phenomenon was believed to be due to a decreased content of sulfhydryl groups in the tumor substrate.[317]

Respiratory enzymes. Investigations dealing with these problems represent a direct approach to the question as to whether, and in what manner, the mechanisms of respiration are affected by neoplasia. The work of the last years has furnished valuable information regarding the steps involved in biological oxidation. A chain of systems participates in the hydrogen transfer from the metabolites to molecular oxygen. Coenzymes I and II (diphosphopyridinenucleotide and triphosphopyridinenucleotide), Warburg's yellow enzyme and cytochromes act alternately as hydrogen acceptors and hydrogen donors until, finally, with participation of indophenol oxidase, the released hydrogen combines with activated oxygen. Evidence has been gathered within recent times to the effect that several of the systems important for biological oxidation are impaired in malignant tumors. Data pertaining to the coenzymes I and II have been given in a previous section of this chapter (cf. p. 500). Here, reference must be made to a few studies dealing with riboflavin, a substance the phosphoric ester of which is the active group of Warburg's yellow respiratory enzyme. As mentioned in chapter V, a diminution of the riboflavin content was ascertained for several animal tumors. Great importance may be also ascribed to the iron-porphyrin compounds which are known as cytochromes (Keilin) and represent very active respiratory catalysts. A considerable decrease of these agents in Jensen rat sarcomas was reported as far back as 1926[318] and analogous results for several other animal tumors were published later.[319] In continued work, transplanted rat and fowl tumors, rat hepatomas induced by butter yellow, and some human neoplasms were tested for these enzymes. Cytochrome *a* was found completely lacking in the tumors, and the quantity of cytochrome *c* was markedly diminished.[320] Extensive studies confirmed the reduced concentration of cytochrome *c* in Jensen sarcomas,[155, 321–323] and the incom-

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[315] K. Vietorisz & A. Szent-Györgyi: Biochem. Ztschr. *240*: 480 (1931).

[316] C. Wetzler-Ligeti & R. Willheim: Ztschr. f. d. ges. exper. Med. *99*: 413 (1936).

[317] R. N. Feinstein & F. J. Stare: Proc. Soc. Exper. Biol. & Med. *45*: 525 (1940).

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318. B. E. Holmes: Biochem. J. *20*: 812 (1926).
319. S. Ito: Tr. Jap. path. soc. *20*: 360 (1930); *21*: 754 (1931).
320. H. Noda: Mitt. a. d. Akad. Med. z. Kioto *20*: 351 (1937).
321. H. v. Euler & H. Hellström: Ztschr. f. physiol. Chem. *255*: 159 (1938).
322. H. v. Euler: Deutsche med. Wchnschr. *64*: 1712 (1938).
323. H. v. Euler, G. Günther & N. Forsman: Ztschr. f. Krebsforsch. *49*: 46 (1939).

pleteness of the cytochrome system was regarded as being responsible for the metabolic alterations and some other pathological phenomena occurring in neoplasms.[322, 323] In satisfactory agreement with the previous work, other investigators also recorded values of cytochrome *c* well below those found in normal organs for various animal tumors (Rous sarcoma of fowl, Kato rabbit sarcoma, mouse and rat tumors).[324] Likewise, low values of cytochrome *c* were determined for rat tumors[324a] and for human liver cancer.[324b] Significant results were obtained in recent studies, according to which the activity of cytochrome systems was definitely lower in tumors than it was found to be in corresponding normal tissues; this alteration was even observed in Shope rabbit papilloma prior to the onset of malignancy.[325] Similarly, analyses of various transplanted and induced tumors of mice, rats and fowl yielded values for cytochrome of 10–20 γ per gram of fresh tissue, i.e., values definitely below those found in most normal tissues.[325a] Thus the disturbance of these oxidative systems may be connected with the problem of malignancy, and further investigation of this question may be expected with great interest.

Definite evidence appears to have been furnished for changes of some other oxidative enzymes, viz., of the activity of dehydrogenases in malignant tumors.[326] It is noteworthy that the activity of these enzymes was found to be severely impaired in vitro by arsenic and by carcinogenic hydrocarbons. These findings were considered as supporting the assumption that a deficiency of dehydrogenases is essentially involved in carcinogenesis.[327] One special factor responsible for the impaired activity of dehydrogenases in tumors was recognized as being the rapid destruction of adenylpyrophosphate—viz., a coferment of dehydrogenases—in tumors. The addition of sufficient amounts of this substance was followed by an activation of the dehydrogenases in malignant tumors.[328] Specific dehydrogenases have been tested in some tumors (cf. chapter IV); the absence of glucose and fructose dehydrogenase was reported for Jensen rat sarcomas.[155] A lactic acid dehydrogenase found in extracts of Jensen sarcomas was shown to act in the same manner as muscle apo-dehydrase. The Jensen sarcomas also contained a malic acid dehydrogenase, but the ratio $\frac{\text{lacticodehydrase}}{\text{malicodehydrase}}$ was found to be higher in the sarcoma than in muscle.[329] It is, moreover, noteworthy that the ratio between reduced and oxidized coenzyme was found to be below unity in normal tissues, whereas the values found in Jensen sarcomas ranged between four and ten.[155, 322] Thus, it was made highly probable that deficiencies of oxidative systems in tumors lead to an accumulation of reducing substances (cf. chapter III). A great number of additional interesting details regarding the hydrogen transfer in neoplasms have been experimentally established.[330, 331] Of special oxidative processes, the combustion of lactic acid in tumor tissue was more precisely investigated. This oxidation is thought to take the following course in normal tissues (muscle): Lactic acid—pyruvic acid (two mols)—succinic acid—fumaric acid—malic acid—oxalacetic acid—pyruvic acid (one mol). Tests as to their possible oxidation were made by means of adding these single intermediate products to tumor slices. Thus, it was shown that in tumors there is a defective oxidation of lactic acid to succinic acid, and the oxidation of succinic acid to fumaric acid as well as the oxidation of malic acid to oxalacetic acid is completely lacking.[332] These relations underwent additional investigation [333-335] and the oxidative metabolism of citric acid was also studied by comparing the reactions of normal and of neoplastic tissues.[336] As mentioned in chapter III, biological reduction has been frequently tested by means of the Thunberg test, which bears mainly on the activity of succinic acid dehydrogenase. Some studies indicated that neoplastic tissues were less active in the reduction of methylene blue than normal tissues.[338, 339] Other authors, however, failed to detect such a difference,[340] or a delayed reduction of methylene blue was observed only in the case of Rous chicken sarcoma, and not in the case of several transplanted rat tumors.[341] Summarizing, it may be stated that studies on dehydrogenases in neoplasms (cf. also chapter IV) have in many instances disclosed an impairment or an alteration of these enzymes which occupy an important place in biological oxidation. Similar conclusions may be drawn from studies in which

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[324] A. Fujita, T. Hata, I. Numata & M. Ajisaka: Biochem. Ztschr. *302*: 376 (1939).

[324a] R. Junowicz-Kocholaty & T. R. Hogness: J. Biol. Chem. *129*: 569 (1939).

[324b] E. Stotz: J. Biol. Chem. *131*: 555 (1939).

[325] F. N. Craig, A. M. Bassett & W. T. Salter: Cancer Research *1*: 869 (1941).

[325a] K. P. DuBois & V. R. Potter: Cancer Research *2*: 290 (1942).

[326] M. Copisarow: Protoplasma (Berlin) *22*: 137 (1934).

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[327] E. Boyland: 1st Internat. Cancer Congress, Madrid *2*: 485 (1933).

[328] E. Boyland & M. E. Boyland: Biochem. J. *29*: 1097 (1935).

[329] H. v. Euler, E. Adler & G. Günther: Ztschr. f. physiol. Chem. *247*: 65 (1937).

[330] A. Szent-Györgyi et al: Ztschr. f. physiol. Chem. *244*: 105 (1936).

[331] F. L. Breusch: Biochem. Ztschr. *295*: 125 (1938).

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332. K. A. C. Elliott, M. P. Benoy & Z. Baker: Biochem. J. *29*: 1937 (1935).
333. K. A. C. Elliott, M. E. Greig & M. P. Benoy: Biochem. J. *31*: 1003 (1937).
334. K. A. C. Elliott & M. E. Greig: Biochem. J. *31*: 1021 (1937).
335. M. P. Benoy & K. A. C. Elliott: Biochem. J. *31*: 1268 (1937).
336. F. L. Breusch: Ztschr. f. physiol. Chem. *250*: 202 (1938).
337. A. H. Drew: Brit. J. Exper. Path. *1*: 115, (1920).
338. H. Heinlein: Ztschr. f. Krebsforsch. *30*: 506 (1930).
339. A. F. Ladeck: Wien. klin. Wchnschr. *46*: 1589 (1933).
340. C. Voegtlin, J. M. Johnson & H. A. Dyer: J. Pharmacol. & Exper. Ther. *24*: 305 (1925).
341. E. S. G. Barron: J. Exper. Med. *55*: 829 (1932).

coctostable respiratory catalysts prepared from normal, or from neoplastic tissues were compared with each other and found to exhibit definite differences. Both boiled muscle and boiled tumor extracts were shown to be capable of oxidizing hexose phosphate. However, the addition of these boiled extracts to Warburg's oxidative system (hexosemonophosphate, plus respiratory ferment prepared from rat blood, plus coferment prepared from horse blood) led to an increased oxygen consumption in the case of the normal tissue extract, whereas the tumor extract caused a diminution of the oxygen consumption. Furthermore, it is significant that an activation of the Pasteur-Meyerhof effect, which can be brought about in Lohmann's muscle extracts by the addition of quinone, was found to be nullified when boiled tumor extract was admixed to the substrate used.[342] Hence, it is conceivable that the coctostable catalysts present in malignant tumors possess qualities distinguishing them from similar factors in normal organs, and these peculiarities may be responsible for the oxidative disturbances apparent in neoplastic tissues.

Respiration of healthy organs of tumor hosts. The velocity of respiration was reported to be decreased in almost all the organs of rats with transplanted tumors, when compared with organs of normal animals.[343] However, the results of Thunberg tests did not reveal any differences between the behavior of muscles of normal and of tumor-bearing rats.[344] On the other hand, the oxygen consumption of boiled extracts of liver, spleen, kidney and muscle was definitely lower when the organs were derived from tumor-bearing animals, as compared with the respective values obtained with organs of animals free of tumors.[316] In a more recent study, the oxygen consumption of liver and abdominal muscle of mice was determined. The organs of animals—both of those free of tumors and of those bearing tumors—yielded lower values when they were derived from animals belonging to a strain of high spontaneous tumor incidence, as compared with the values in organs of mice belonging to a low-tumor strain.[345] These findings are doubtless very interesting, yet the evidence available is not sufficient to warrant the assumption that the disturbance of respiration and oxidation is a fundamental factor in a predisposition to, or in the development of, neoplasia. In particular, reference is made to a study previously discussed, according to which the oxidative metabolism of mice treated with benzpyrene is not affected prior to the morphological appearance of tumors.[184]

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[342] C. Wetzler-Ligeti & R. Willheim: Ztschr. f. d. ges. exper. Med. *99*: 416 (1936).

[343] S. M. Neuschlosz: Klin. Wchnschr. *3*: 57 (1924).

[344] B. Purjesz, L. Berkessy & K. Gönczi: Ztschr. f. Krebsforsch. *41*: 358 (1935).

With regard to the blood, the most important oxygen-transporting medium, it is recalled that Warburg, on the basis of metabolic determinations and of theoretical oxygen requirements, calculated that practically the total oxygen content of the blood supplied to the tumors is needed for the combustion of carbohydrates. It is therefore especially interesting to refer to experimental work, according to which the venous blood of tumor-bearing extremities possessed a higher oxygen content—expressed as percentage of the oxygen content of the arterial blood—than was the case in venous blood of normal extremities.[346] This may suggest a reduced oxygen consumption in the tumor and perhaps also in the adjacent normal tissue. However, it cannot be decided whether this phenomenon is caused by characteristic qualities of neoplastic tissues, or whether it is merely a secondary con-

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345. J. E. Davis: Canad. M. A. J. *36*: *27* (1937).

346. A. Oszacki: Bull. internat. Acad. Polsk. Sc., Cl. Med. *4*: 169 (1932); abstr. Ztschr. f. Krebsforsch. *39*: Ref. 19 (1933); 1st Internat, Cancer Congress, Madrid *2*; 73 (1933).

sequence of local circulatory disturbances. Conflicting statements—probably on account of differences in the techniques used—were made as to the presence of respiratory catalysts or coferments in sera of tumor hosts. A report of older date claimed that the oxygen consumption of muscle cells, as measured by the reduction of dinitrophenol, was increased by sera of cancerous persons, but not by sera of normal persons.[347] Another worker, however, found no difference between normal and cancerous sera, both of which were seen to stimulate the oxygen consumption of muscle.[348] In complete contrast to these findings, more recent investigations reported that sera and serum ultrafiltrates of tumor patients activated the respiration of tissue slices in vitro to a much smaller extent than sera of normal persons.[349, 350]

Another trend of work was based on the previously described observation that the glycolysis of the blood of tumor patients was, in contrast to the reaction of normal blood, lowered by the addition of boiled muscle extract. This phenomenon was interpreted as being due to a stimulation of the Pasteur effect and, accordingly, the following theoretical inference was possible. The boiled muscle extract may contain oxidation catalysts which replace or activate principles which are either absent or inactive in the erythrocytes of tumor hosts, and which are required for the Pasteur effect. This hypothesis was supported by the results of direct determinations of the oxygen consumption. When washed red blood cells were hemolyzed and their respiration was measured, (a) in the absence, and (b) in the presence of the boiled muscle extract, an increased oxygen consumption was observed in the latter instance, provided that the eryth-

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347. S. M. Neuschlosz: Klin. Wchnschr. *3*: 628 (1924).
348. A. Simon: Klin. Wchnschr. *4*: 499 (1925).
349. L. Karczag: Ztschr. f. Krebsforsch. *39*: 23 (1933).
350. B. Walthard: Ztschr. f. d. ges. exper. Med. *95*: 682 (1935).

rocytes were derived from tumor hosts. This effect, however, did not take place in experiments performed with hemolysates derived from persons free of neoplasia.[351] The utilization of these findings for diagnostic purposes was considered (cf. chapter X). At this place, however, emphasis is laid on the theoretical significance of the results described, inasmuch as they speak in favor of the assumption that the red blood cells of tumor hosts contain incomplete respiratory systems, which can be supplemented by catalysts derived from normal organs.

Clues for a disturbance of the oxidation in tumor hosts were believed also to be furnished by the results of the analyses of urines. This refers to determinations of the ratio carbon/nitrogen in the urine, i.e., the proportion between the excretion of oxidisable carbon compounds and of nitrogenous metabolites. An increase of this ratio is termed "dysoxidative carbonuria." As a matter of fact, it was reported that the normal ratio averaging 0. 7 is contrasted with values in tumor patients ranging from 0.9 to 2.7.[352-354] These findings were believed to indicate a diminished combustion in the bodies of tumor hosts, but the small amount of cases tested, and the insufficient theoretical elaboration do not as yet warrant the drawing of definite conclusions.

Experimental modification of tumor respiration. In analogy to the work with glycolysis, the studies in this field were chiefly aimed at detecting different reactions of normal and of neoplastic tissues in response to the same agents. In satisfactory agreement with Warburg's statements discussed

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351. C. Wetzler-Ligeti & R. Willheim: Ztschr. f. d. ges. exper. Med. *99*: 422 (1936).

352. A. Bickel & O. Kaufmann-Cosla: Virchow's Arch. f. Path. *259*: 186 (1926).

353. N. Medvedewa: Deutsche med. Wchnschr. *53*: 1732 (1927).

354. D. Kritchin: Med. Ž. vseukrain Akad. *6*: 1111 (1936); abstr. Ztschr. f. Krebsforsch. *46*: Ref. 241 (1937).

in the section on carbohydrate metabolism, the respiration of Jensen rat sarcomas was found to be independent of the presence of glucose. In contrast to this, the respiration of most normal tissues is severely impaired when they are deprived of glucose, and the respiration of embryonic tissue is lowered under these circumstances.[355] An analogous reaction of normal and of neoplastic tissues was observed, inasmuch as in both substrates the respiration was in part found resistant and in part sensitive to the addition of arsenic, or cyanide.[356] Normal organs of mice and rats and transplanted mouse carcinomas and rat sarcomas were compared in extensive studies concerned with the influence of several agents on their respiration. Marked differences were disclosed in this manner: most of the substances stimulating the respiration of normal tissues, such as the addition of aluminum chloride, calcium chloride, sodium nitrite, a number of amino acids[357] and of salts of fatty acids,[358] were ineffective in tumors. A stimulation of the tumor respiration was achieved by lactates and pyruvates,[358] and by the amino acids serine and valine, which substances were ineffective in normal tissues.[359] An increase of the respiration of rat sarcomas was observed by other workers after the addition of fumarates and succinates, while malonates exerted an inhibiting effect.[360] These effects correspond to those observed in normal guinea pig muscle.[361] It is of interest that retina, which has a similar glycolytic activity as tumor tissue, was clearly distinguished from neoplastic tissue in regard to the influence on its respiration.[362] Embryonic tissue, on the other hand, exhibited an intermediate position between neoplastic and normal tissue.[363] The sensitivity to cyanide of the tumor respiration was, according to this report, considerably greater than that of many normal tissues.[364] The respiration of kidneys of tumor-bearing rats was observed to react in a manner differing from that of organs of normal animals to the previously enumerated agents.[365] This may support the assumption of systemic alterations of the metabolism in neoplasia. A significant observation was made in that the oxygen consumption of tumor tissue is regularly increased after the addition of methylene blue, whereas such an effect occurs in normal tissues only in the case of a previous poisoning with cyanide.[366] According to these results, the tumor acts, to start with, as a biological substrate which is damaged in its respiratory qualities. Barium salts, particularly the gluconate and the saccharate, the chloride less so, were reported to promote the respiration of cancerous tissue.[367] These experimental findings were regarded as warranting the employment of barium salts in the combined organotherapy developed by Maisin and his school (cf. chapter VIII). Carcinogenic hydrocarbons, on the other hand, were claimed to lower the respiration in vitro,[368] but in a recent study benzpyrene, at least, was found to be without effect on the oxygen consumption both in vivo and in vitro,[184] and, accordingly, the involvement of an impaired oxygen consumption in carcinogenesis is not yet clarified.

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[355] O. O. Bogomoletz: Med. Ž. vševkrain Akad. Nauk. *4*: 1 (1934); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 263 (1935).

[356] F. Dickens & G. D. Greville: Biochem. J. *27*: 832 (1933).

[357] B. Kisch: Biochem. Ztschr. *237*: 226 (1931); *238*: 364 (1931).

[358] B. Kisch: Biochem. Ztschr. *253*: 379 (1932).

[359] B. Kisch: Biochem. Ztschr. *247*: 354 (1932).

[360] E. Boyland & M. E. Boyland: Biochem. J. *30*: 224 (1936).

[361] A. Szent-Györgyi: Ztschr. f. physiol. Chem. *236*: 1 (1935).

Several organic compounds were tested as to their effect

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362. B. Kisch: Biochem. Ztschr. *244*: 459 (1932).
363. B. Kisch: Biochem. Ztschr. *238*: 370 (1931).
364. B. Kisch: Biochem. Ztschr. *263*: 75 (1933).
365. B. Kisch: Biochem. Ztschr. *273*: 324 (1934).
366. E. S. G. Barron: J. Exper. Med. *52*: 447 (1930).
367. Y. Pourbaix: 1st Internat. Cancer Congress, Madrid *1*: 72 (1933).
368. Y. Pourbaix: 1st Internat. Cancer Congress, Madrid *2*: 255 (1933).

on respiration and on the glycolysis of tumors. Thionine added to rat kidney or to rat sarcomas caused an increase of the oxygen consumption by fifty to ninety per cent and, at the same time, the aerobic glycolysis disappeared completely.[241] In later work, a stimulation of the respiration in transplanted rat and mouse tumors by thionine and by brilliant cresyl blue was seen.[369] The addition of dinitrophenol-o-cresol to tumor slices caused an increase both of respiration and of glycolysis.[242] A stimulation of the oxygen consumption was also achieved by the addition of pyocyanin.[370] Yet, in this connection it is interesting that this substance was reported to enhance the carcinogenesis in mice painted with benzpyrene,[371] whereas another respiration catalyst—thionine—was found to be without influence on the tumor formation induced in mice by benzyprene.[372] In agreement with an older report,[366] methylene blue was shown to activate the respiration of tumor much more markedly than it activates that of normal tissues. It is, however, noteworthy that this compound in normal tissues raised the respiratory quotient, whereas the respiratory quotient of tumors mixed with methylene blue was lowered. Specifically, the increased oxygen consumption cannot be connected with an increase of complete combustion of carbohydrates and of lactic acid.[373] A different response of normal organs and of malignant tumors was observed in regard to the effect of 2, 6-dichlorophenol indophenol. While the respiration of most normal organs was inhibited by this compound both in the presence and in the absence of glucose, tumors showed an increased oxygen consumption when adequate amounts of the compound were added together with glucose. In normal organs,

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369. F. Dickens: Biochem. J. *30*: 1065 (1936).
370. E. A. H. Friedheim: Biochem. J. *28*: 173 (1934).
371. W. Beltrami: Tumori n.s. *14*: 41 (1940).
372. W. Beltrami: Tumori n.s. *12*: 205 (1938).
373. J. J. Jares: Am. J. Cancer *24*: 80 (1935).

only hepatic tissue reacted in this manner. Identical effects were observed of a number of organic dyes possessing a reduction-oxidation potential similar to dichlorophenol indophenol.[374] Hematoxyline was reported markedly to increase the respiration of rat carcinomas and also of the spleen of tumor-bearing animals, whereas the respiration of organs of animals free of tumors was lowered by this compound.[375]

Disturbances of oxygen consumption and tumor etiology. As discussed in the section on carbohydrate metabolism, Warburg's "selection theory" held that, under conditions of inadequate oxygen supply, only those cells could survive which, endowed with high glycolytic activity, are independent of oxidative processes and can cover their energy requirements by fermentation. These cells, exhibiting the metabolic properties of cancer cells, were believed, in addition, to have acquired other characteristics of neoplastic tissue. This interesting theory as well as similar trends of thought previously mentioned have so far not found definite support by experimental work. On the contrary, a group of authors believed they had evidence for the occurrence of an opposite phenomenon, i.e., of an increased oxygen consumption accompanying the first stages of carcinogenesis. This claim was based on investigations performed with rabbits, the ears of which were painted with tar or treated with other carcinogens. Blood gas analyses and determinations of the dehydrogenase activity were reported to show that, starting with the inflammatory reaction until the morphological appearance of neoplasia, the oxidative processes in the treated tissues are definitely increased.[376, 377] Consequently, it was thought justifiable to regard an increased oxygen consumption as being basically associated with carcinogenesis. This, it was reasoned, represents a factor favorable for protein synthesis and accordingly for the intensive neoplastic growth rate. On the other hand, it was stated that "the glycolytic and proteolytic cleavage processes represent merely the negative side of the tumor riddle," obviously caused by the secondary disintegration of neoplasms.[378] Nevertheless, it appears questionable as to whether the evidence presented warrants these far-reaching conclusions and, in particular, a study previously mentioned led to definitely divergent results; that is, investigations of mice treated with benzpyrene did not show any measurable effect of the carcinogen on the oxidative processes prior to the onset of malignancy.[184] It is possible that the increased oxygen consumption, observed in the aforementioned studies,[376-378] was in reality due to the inflammatory reactions and not to the development of neoplasia. At any rate, a continued investigation into the oxygen metabolism during carcinogenesis represents an approach which will perhaps contribute to the understanding of this pathology. A theory of a speculative nature attempted in a novel way to connect tumor etiology with disturbances of oxidation; namely, it was presumed that a faulty mechanism of dehydrogenation would lead to a conversion of sterols or bile acids to compounds related to the carcinogenic hydrocarbons. Accordingly, it was believed that the administration of substances which act as oxygen acceptors might prevent this dehydrogenation, and thus might impede the development and growth of tumors.[379] Support for this assumption was seen in some observations of other authors, such as an inhibition of tumor growth after the administration of garlic which contains unsaturated allyl compounds,[380] or after the administration of thiosinamine.[381] It was proposed to undertake experiments with similar compounds which intercept oxygen (e.g., anethol) and thus to prevent the assumed dehydrogenation of sterols,[379] but so far no announcements have been made which prove the effectiveness of such measures.

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[374] K. A. C. Elliott & Z. Baker: Biochem. J. *29*: 2396 (1935).
[375] R. Iwatsuru & M. Kawaguchi: Biochem. Ztschr. *284*: 163 (1936).
[376] R. Deotto: Tumori n.s. *10*: 77 (1936).
[377] D. V. Bori: Tumori n.s. *10*: 223 (1936).

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[378] P. Rondoni: Ztschr. f. physiol. Chem. *235*: 81 (1935); Acta Cancrol. *2*: 205 (1936).

[379] A. Müller: Österr. Chem. Ztg. *40*: 305 (1937); Wien. klin. Wchnschr. *50*: 1614 (1937).

Therapeutic measures and oxidative metabolism. The attemps to counteract neoplasia by modifying the oxygen consumption are in part connected with therapeutic measures, enumerated previously, which aim at a suppression of glycolytic processes in the tumor tissues. Accordingly, these experiments are subject to the same limitations discussed before, and reference is made to theoretical objections which were raised against the rationality of considering a change of metabolic properties as a practicable approach to cancer therapy.

An indirect involvement of respiration was assumed to exist in regard to radiation therapy as used in cancer treatment; that is, it was shown that the radiosensitivity of malignant tissue in vitro increased proportionally with the impairment of its respiration as caused, for instance, by cyanide or by low temperatures,[382] and similar opinions were expressed by other authors.[383] It was assumed that, in vivo, the effect of irradiation of tumors is probably increased in the case of poor vascularization and insufficient oxygen supply.

Recently, animal experiments were performed to test the effect of local asphyxia on transplanted tumors (Crocker sarcomas, Flexner-Jobling carcinomas, Walker tumors of rats). Repeated ligations, of short duration, of extremities, into which the tumors had been inoculated for not too long a period, caused hemorrhages of the tumor vessels and consequent sloughing out of the tumors. The normal tissue did not appear to be damaged by this treatment, and the effect on the neoplastic tissue was considered to be a specific vascular reaction. However, visceral tumors were affected far less favorably and, generally, in spite of necrosis of the tumors no permanent cure could be achieved by the treatment outlined.[384] Likewise, further efforts to support the asphyxia therapy by the administration of lead or arsenic preparations only led to incomplete and inconstant results.[385] It may be recalled that the action of metals used for tumor therapy was shown to be accompanied by severe vascular damages (cf. chapter I), but apparently this curtailment of the oxygen supply of neoplasms is not sufficient to bring about their complete and permanent destruction. Analogous results were obtained in experiments with rat sarcomas induced by means of injections of methylcholanthrene into the hindlegs of the animals. Complete mechanical arrest of the circulation in the tumor-bearing limb was performed for six to seven hours. In consequence of this interference, extensive central necrosis of the tumors set in, but viable tumor cells were always found to persist in the periphery. In spite of the fact that tumor tissue appeared to be more sensitive to restriction of the blood supply than normal tissues, it had to be concluded that this measure is not capable of curing tumors.[385a]

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[380] W. Caspari: Ztschr. f. Krebsforsch. *43*: 255 (1936); Nutrition et Cancer, Herman & Cie., Paris 1938.

[381] H. Koenigsfeld & C. Prausnitz: Deutsche med. Wchnschr. *39*: 2466 (1913).

[382] W. Cramer: 1st Internat. Cancer Congress, Madrid *2*: 175 (1933).

[383] J. Firket, J. Lambert & M. Chèvremont: 1st Internat. Cancer Congress, Madrid *2*: 208 (1933).

Another approach made use of direct changes in the gas supply available for respiration. In experiments with tissue cultures, oxygen at a pressure increased to five atmospheres was applied to the cells, and this treatment was reported to destroy sarcomatous cells earlier than it did normal fibroblasts.[386, 387] However, animal experiments employing compressed oxygen did not yield conclusive results. One worker found that transplanted rat tumors were cured in seven to twenty-three per cent of the cases, when the animals were exposed to pressures of two to eight atmospheres of oxygen. The mortality, however, of the animals was very high.[388] Moreover, a re-examination of this treatment failed to confirm the earlier report, i.e., rats with Walker tumors, mice with Twort or with Bashford carcinomas were treated at pressures of four to five atmospheres of oxygen, but the growth of the tumors was not inhibited—instead, it was, at times, even accelerated.[389] The difficulty of destroying tumor cells by high pressure was also demonstrated by a study, according to which tumor cells were still transplantable after having been exposed to fifty-five atmospheres of carbon dioxide, to ninety atmospheres of oxygen, or to 120 atmospheres of nitrogen.[390]

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[384] F. M. Allen: Urol. & Cutan. Rev. *44*: 12 (1940); Am. J. Surg. *49*: 484 (1940); Arch. Surg. *41*: 79 (1940); Am. J. Clin. Path. *10*: 813 (1940).

[385] F. M. Allen: J. Lab. & Clin. Med. *26*: 1120 (1941).

[385a] P. Salzburg & H. Kabat: Arch. Surg. *42*: 917 (1941).

On the other hand, a reduction of the atmospheric pressure or of the oxygen content has been tried as a possible means for tumor therapy.[391, 392] However, experiments with tissue cultures showed that a reduction of the oxygen content, or an increase of the carbon dioxide content, damaged neoplastic cells to a lesser degree than it did normal cells.[393]

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[386] A. Fischer & E. B. Andersen: Ztschr. f. Krebsforsch. *23*: 12 (1926); Compt. rend. Soc. de biol. *93*: 1677 (1925).

[387] A. Fischer, E. B. Andersen, F. Demuth & H. Laser: Ztschr. f. Krebsforsch. *24*: 528; 563 (1927).

[388] O. de Almeida: Compt. rend. Soc. de biol. *116*: 1125; 1228 (1934).

[389] J. A. Campbell: Brit. J. Exper. Path. *18*: 191 (1937).

[390] H. Auler: Libro de Oro, Buenos Aires, p. 56 (1935).

[391] H. Auler, H. Herzogenrath & B. Wolff: Ztschr. f. Krebsforsch. *28*: 466 (1929).

[392] J. A. Campbell & W. Cramer: Lancet *1*: 828 (1928).

[393] J. C. Mottram: Lancet *2*: 1232 (1927).

Later work supplemented these data by showing that exposure to a reduced oxygen pressure did not affect the respiration of tumors in vitro, but increased the aerobic glycolysis.[394] More recently, it was disclosed that lowering of the atmospheric pressure, and reducing of the oxygen content to fifty per cent for three weeks, failed to influence the growth of mouse sarcomas 180 and of spontaneous mouse tumors.[395]

A very complex therapy aimed at an intensification of respiration and oxidative metabolism in neoplasia was devised and employed by Fischer-Wasels.[181, 396] The essential part of this treatment was a gas therapy, which consisted of exposure to a gas mixture with increased oxygen and carbon dioxide concentrations. In addition, the administration of copper and iron (respiratory catalysts), of hormones (thyroxine) and of organic compounds stimulating the oxygen consumption (glyceric aldehyde, dioxyacetone) was employed. Animal experiments were reported to prove an effectiveness of this combined treatment, and some clinical experiences were also regarded by the author as being encouraging. An interpretation of the latter results, however, is rendered difficult by the circumstance that the "gas therapy" was also accompanied by irradiation of the patients. Only few other investigators tested Fischer-Wasels' therapeutic proposals, and the results recorded are either only slightly favorable [397, 398] or completely negative.[399] These circumstances, and the complicated equipment required for the therapy (special gas chambers), make it understandable that these experiments have since been abandoned.

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394. H. Laser: Biochem. J. *31*: 1671 (1937).
395. G. L. Rohdenburg: Cancer Research *1*: 310 (1941).
396. B. Fischer-Wasels: Klin. Wchnschr. *7*: 53; 106; 153 (1928).
397. H. Kahler: Wien. klin. Wchnschr. *46*: 1404 (1933).
398. M. Tanaka & K. Muto: Gann *29*: 88 (1935); abstr. Am. J. Cancer *25*: 662 (1935).
399. Ch. C. Lund & H. M. Hulton: Am. J. Cancer *16*: 1489 (1932).

The therapeutic measures discussed in the preceding section were principally aimed at modifying the external conditions of the oxygen consumption. Apparently, they have so far failed to achieve definite successes, a fact which may be interpreted either as indicating that the disturbances of the tumor respiration are not essentially connected with the neoplastic process, or that the approaches chosen were not the right ones. As to the latter assumption, it may be recalled that many facts clearly pointed to intracellular deficiencies of enzymatic systems or of other catalysts as being responsible for the impairment of oxidative processes in neoplastic tissue. Hence, it may be understood why changes in the gas supply, offered to tumor hosts for respiration, must fail to restore these pathological alterations in tumor cells to normal. A greater success may be expected from a direct approach aimed at relieving the intracellular deficiencies of malignant tumors —difficult as such an undertaking may appear. Only few attempts of this kind have thus far been made, such as in those previously enumerated endeavors to isolate activators of respiration from normal biological substrates.[267, 268] Perhaps there is a relation between the problem under discussion and between experiments which employed high doses of nicotinic acid in the treatment of certain cancer cases.[400] As described in chapter V, this procedure led to some significant results, particularly in cancers of the oral cavity; the authors of this study called attention to the fact that nicotinic acid is regarded as an important constituent of respiratory catalysts (coenzymes), and may accordingly act by restoring a deficiency of this factor assumed to exist in neoplastic cells.[400] Actually, ingestion of nicotinic acid has been demonstrated as being followed by a conversion to coenzyme, and as causing an increased oxygen consumption.[401] Doubtless

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400. R. Willheim & F. Bocobo: Acta med. Philipp. *2*: 445 (1941).
401. H. I. Kohn, J. R. Klein & W. J. Dann: Biochem. J. *33*: 1432 (1939)

the evidence so far available must be considered preliminary, but it does represent a definite incentive for continued endeavors in this direction.

Chapter VII

ENDOCRINE GLANDS AND THEIR HORMONES

While originally arrayed with physiology and clinical pathology, endocrinology has now also become an integral part of physiological and pathological chemistry. This fact is due to the concerted efforts of laboratory research in which the chemical isolation of pure hormones and their structural identification have been accomplished in most cases, and their synthetic preparation has, in many instances, been achieved. Hence the chemistry of neoplasia necessarily must include the interrelationship between hormones or their source, the endocrine glands, and malignant tumors. This interdependence acquires an increased significance when the fact is recognized that endocrines exert a regulating or determining influence on a multitude of physiological processes, among them growth and metabolism. Alterations of hormonal mechanisms have therefore been suspected as being involved etiologically in neoplasia, or have been supposed to be associated, at least, with these diseases. There will, in the following, be several occasions to discuss even more far-reaching attempts to achieve therapeutical gains by the administration of certain hormones, or by the impairing of the internal secretion of one gland or the other.

The subdivisions of this chapter, devoted to the discussion of the individual glands, are arranged in such a way that the morphological aspects of the various endocrines in neoplasia will be surveyed first, then the quantitative and qualitative relations of their secretions will be considered, and finally those studies will be presented in which the effect of the administration or exclusion of certain endocrine factors on tumor development and tumor growth has been investigated. It may be anticipated that many conflicting statements will be encountered in this regard, that is, in many instances, extirpation of a gland, or administration of its hormone, has been claimed by some authors as inhibiting the tumor process, whereas other investigators interpreted their experimental observations in an opposite manner. For these divergencies, differences in the experimental arrangements—use of inoculated, spontaneous, or chemically induced animal tumors, or tests performed with tissue cultures, or studies on clinical material—are partially responsible. In addition, the endocrine preparations employed frequently varied as to their purity and as to the incidental admixture of other factors. Last, but not least, it must always be kept in mind that the administration, or withdrawal, of one hormonal factor may cause a disturbance of the extremely sensitive equilibrium prevailing in the body and give rise to unexpected compensatory mechanisms, thus obscuring the effect of a planned interference.

1. *The Thyroid Gland.*

Morphological findings. An atrophy, or degeneration of the thyroid observed in young persons afflicted with cancer has been interpreted as an anatomical indication of an alleged antiblastic activity of this gland.[1] Animal experiments showed that transmission of a sarcoma to rabbits was followed about twenty days after the tumor onset by an enlargement of the thyroid follicles, resulting later in an atrophy of the epithelial lining.[2] This reaction does not appear to be shared by animals inoculated with other types of neoplasms, since no appreciable changes of the thyroid morphology were found in mice after the transplantation of a lymphosarcoma, whereas mice affected with spontaneous tumors were reported to exhibit a hyperactivity of the thyroid histologically.[3] Since, however, this hyperactivity could be made to disappear by means of thyroxine injections, as is possible in many cases of struma developments but without any noticeable influence on the tumor growth,[4] it is obvious that this phenomenon was not primarily connected with the neoplastic process, but represented rather a secondary consequence of the disease. In this connection, it may be recalled that the basal metabolic rate of some cancer patients has been found increased, a finding related to the thyroid activity, but no characteristic significance could be assigned to it, as mentioned in chapter VI. Nevertheless, some authors seemed to view this hyperthyroidism as a defense reaction of the organism against cancer and, in support of this assumption, it was pointed out that tumor incidence among patients with Graves' disease should be very rare.[5] However, neither these statistical claims, nor the histological findings can be regarded as sufficiently strong evidence for such a theory.

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[1] A. Lorand: 1st Internat. Cancer Congress, Madrid *2*: 48 (1933).

Thyroid secretion. These relations have been the subject of only very few studies, in one of which the thyroxine content in the serum of cancer patients has been assayed by means of the Reid-Hunt test, i.e., the decreased toxicity of acetonitrile in mice following thyroxine administration. No deviation from the normal was found in neoplasia, as judged from the effect of cancerous serum.[6] The finding of an antithyrotropic quality in about seventy-five per cent of tested cancer sera[7] might be taken as indication of an alteration directed against the action of a pituitary factor which activates the thyroid function. Later on, other antihormonal factors which counteract pituitary secretions will be mentioned.

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[2] S. Nishida: Jap. J. Obst. & Gynec. *18*: 195 (1935).

[3] L. Mercier & L. Gosselin: Compt. rend. Soc. de biol. *116*: 30; *117*: 762 (1934).

[4] L. Mercier & L. Gosselin: Compt. rend. Soc. de biol. *118*; 17 (1935).

[5] G. Domagk: Ztschr. f. Krebsforsch. *44*: 160 (1936).

Influence of thyroid on tumor process. Several factors, known from normal physiology, may serve as a theoretical basis for expecting that the thyroid secretion could cause changes in the course of the tumor growth. On the one hand, reference is made to the promotion of cell differentiation exerted by the thyroid gland and its hormone, as evidenced by the familiar tadpole experiments, and it could be assumed that this endocrine factor might correspondingly exert a similar influence on neoplasms, i.e., may counteract the lack of differentiation apparent in tumor tissue, and thus reduce the grade of malignancy.[8] Actually, an inhibition of tumor growth, following peroral or parenteral thyroxine administration, has been reported by some investigators,[9-12] one of whom saw in such experiments a true antagonism between this effect of thyroxine and the tumor promoting influence of arginine,[13] (cf. chapter II), and, on the other hand, thyroidectomy was claimed to stimulate the growth of animal tumors.[14] In consequence of these results of animal experimentation, clinical therapeutic attempts have been undertaken by implanting thyroids, especially hyperactive glands (Graves' disease), into inoperable cancer patients and beneficial effects were attributed to this treatment.[15] In addition to the influence on cell differentiation, an alteration of the tumor metabolism has been considered as furnishing the basis for a possible effect of the thyroid on neoplasia, proof for which assumption has been seen in an investigation in which the metabolic processes were measured in the tissues of animals previously treated with thyroxine in vivo, whereas under these conditions the oxygen consumption of normal tissue was found markedly increased, that of tumors was appreciably lowered (up to twenty-five per cent) ; no alteration of the glycolysis was found in normal or neoplastic tissues.[16] In interpreting these findings, it would be possible to assume that this impairment of the oxygen intake of tumors, caused by thyroxine, might decisively damage their vitality, since their oxygen supply a priori seems to be insufficient (cf. chapter VI). But continued investigations of this problem revealed that the relations in question are far more complicated than was apparent from the findings enumerated thus far, which seemed to imply a tumor inhibiting influence of the thyroid hormone. To begin with, the factor of dosage seems to be of importance, since it was reported that the growth of an inoculated rabbit sarcoma was promoted by small thyroxine doses, whereas administration of larger amounts impeded the tumor process.[17] Similarly, a slight retardation of transplanted mouse tumors could be achieved only under special conditions, involving injections of comparatively large amounts of thyroxine starting in the first stages of the tumor development.[18] In contrast to a claim mentioned above,[14] a number of studies indicated an inhibition of tumor growth after thyroidectomy of tumor-bearing animals.[2, 17, 19-21] An analogous procedure performed in cancer patients yielded results which cannot be regarded as convincingly proving the therapeutic value of thyroidectomy in these cases.[22] The growth inhibition of animal tumors following thyroidectomy has been explained as being a secondary effect, due to an impairment of the pituitary function, since administration of the hypophysial growth hormone, about which more will follow later, counteracted the inhibitory influence of thyroidectomy.[21] Other authors, who observed a slight growth stimulation of transplanted rat sarcomas in animals subjected to extirpation of their thyroid and parathyroid glands and which were fed thyroxine, likewise considered this effect as not specific, but as being due to an improvement of the general condition of the animals.[23] These complex relations—as expressed in the fact that every interference with one endocrine organ may upset the interrelationship with other hormonal factors—may be responsible for the discrepancies mentioned as well as for those reported in other studies in which thyroxine administration had only an inconstant influence on tumor growth,[2] failed to affect it at all,[4, 24, 25] or inhibited the tumor process only if the dosage employed resulted in a loss of the total body weight of the animals.[26] The opposite effect—stimulation of tumor growth—seemed to be indicated by experiments with tissue cultures of Jensen rat sarcoma, the growth of which was promoted by

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[6] S. Sommer: Zentralbl. f. Gynäk. *58*: 385 (1934).
[7] H. Eitel: Klin. Wchnschr. *17*: 1465 (1938).
[8] A. Lumière: 1st Internat. Cancer Congress, Madrid *2*: 1008 (1933).
[9] R. Honda: Tr. Jap. Path. Soc. *14*: 289 (1924).
[10] E. Gilroy: Biochem. J. *24*: 589 (1930).
[11] W. Weichardt: Ztschr. f. d. ges. exper. Med. *90*: 279 (1933); Klin. Wchnschr. *12*: 1060 (1933).
[12] O. O. Meyer & C. McTiernan: Am. J. Cancer *20*: 96 (1934).
[13] E. Gilroy: Biochem. J. *24*: 1181; 1659 (1930).
[14] V. Korentchevsky: Compt. rend. Soc. de biol. *83*: 779 (1920).

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[15] F. Burgkhardt: Med. Welt *7*: 1355 (1933).

[16] O. O. Meyer, C. McTiernan & J. C. Aub: J. Clin. Investigation *12*: 723 (1933).

[17] N. Murohara: Tr. Jap. Path. Soc. *20*: 658 (1930).

[18] W. Moppett: Med. J. Australia *24*, *2*: 1065 (1937).

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19. J. Flaks: Ztschr. f. Krebsforsch. *27*: 548 (1926).
20. M. Levine & V. H. Kugel: Am. J. Cancer *19*: 817 (1933).
21. M. Reiss & J. Balint: Med. Klin. *30*: 706 (1934).
22. W. Stuart-Low: Lancet *2*: 1138 (1909).
23. R. L. Ferguson, R. D. Templeton, M. C. Petras & F. A. McJunkin: Arch. Path. *29*: 785 (1940).
24. F. Bischoff, M. L. Long & L. C. Maxwell: Am. J. Cancer *24*: 549 (1935).
25. F. F. Beck & J. C. Krantz, Jr.: Cancer Research *1*: 188 (1941).
26. F. Bischoff & M. L. Long: Am. J. Cancer *31*: 67 (1937).

the addition of thyroid extract,[27] and by a study in which the formation of tar cancer in mice was found accelerated when thyroid substance was fed to the animals.[28] Nutritive influences have also been tested, in the form of the Reid-Hunt diets, which either activate or decrease the thyroid secretion, and it was reported that the effect on tumor growth corresponded to that on the thyroid gland, i.e., increased or decreased glandular function was accompanied by a tumor stimulation or a retardation.[29] Later studies, however, indicated that an inhibition of mouse and rat tumors, observed in animals fed a diet which produced strumas, depended merely on the general health debilitation of the animals.[30] It is obvious, from the experimental results presented, that no uniform influence of the thyroid secretion on the neoplastic process has been established, and that the effects observed probably are determined not only by the kind and the amount of the preparation used, but also by dietary factors and by the interrelationship with other endocrine glands, which are interfered with in the experiments and which may affect the general condition of the animals. Hence no serious consideration can be given to a far-reaching theory which claimed by means of statistical analyses to have established a parallel incidence of cancer and goiter and consequently regarded neoplasia as being caused by thyroid insufficiency.[31]

2. *The Parathyroid Glands.*

A connection between these endocrine organs and neoplasia was suggested principally on account of their regulating function on mineral metabolism, especially by means of mobilizing calcium in the body. Calcium has been considered by many authors as antagonizing the tumor process, but in addition to the disputed evidence of the tumor inhibiting quality of calcium, as mentioned in chapter I, the studies on the parathyroid glands in neoplasia have been hampered by difficulties arising from an insufficient knowledge of the physiology of this gland, and also by the lack of uniform preparations of its hormone, the chemical configuration of which has not yet been determined.

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[27] Z. Zakrzewski: Ztschr. f. Krebsforsch. *30*: 122 (1929).

[28] L. Kreyberg: Acta path. et microbiol. Scandinav., Suppl. *37*: 317 (1938).

[29] E. P. Corson-White & G. J. Saxon: J. Biol. Chem. *15*: 181 (1913).

[30] K. Sugiura & S. R. Benedict: Am. J. Cancer *23*: 541 (1935).

[31] W. M. Stevens: Cancer, its causation, prevention and treatment, Lewis & Co., London 1935.

Morphological findings. An investigation of thirty cancer patients seemed to demonstrate the presence of a parathyroid insufficiency, evidenced by a structural damage, a decreased number of glands, and an impaired functional activity.[32] An insufficiency of the parathyroid function has also been believed to be responsible for the increased guanidine content in the serum of cancer patients [33] (cf. chapter II). Histologic changes of fibrous and hyaline nature have also been observed in the parathyroids of mice with tar cancer or spontaneous tumors, and it was stressed that these regressive alterations in the glands failed to occur in animals resistant to tumor development.[34]

Influence on tumor growth. A number of studies yielded results suggesting an inhibition of animal tumors as a consequence of the administration of parathyroid extracts, and this effect was regarded as being due to the calcium augmentation brought about by this treatment.[35] More recently, an inhibition of Jensen rat sarcoma, following parathyroid hormone injections, has been reported [36] and the growth of inocu-

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32. P. A. Kutscherenko & R. M. Maislisch: Acta med. Scandinav. *85*: 89 (1935).

33. J. Ducuing, F. H. Colombies & J. Lansac: 1st Internat. Cancer Congress, Madrid *2*: 503 (1933).

34. L. Th. Larionow: Vestn. Rentgenol. *16*: 237 (1936); Bull. Assoc. franç. p. l'étude du cancer *27*: 628 (1938).

35. M. Goldzieher & E. Rosenthal: Ztschr. f. Krebsforsch. *13*: 321 (1913).

36. J. H. Thompson: Brit. M. J. *1*: 819 (1931).

lated rabbit sarcoma was found to be impeded after the administration of this hormone, while the opposite effect occurred in parathyroidectomized animals, even if only a partial extirpation had been performed.[37] General recommendations of a parathyroid therapy in clinical cases have been advanced,[38] but some experiences led to unclear results [36] and, furthermore, additional animal experimentation yielded conflicting findings, such as growth stimulation of rat carcinomas by parathyroid hormone administration,[39, 40] or led to the observation of regressions of transplanted rat sarcomas after extirpation of the parathyroid and thyroid glands.[41] Finally, the use of parathyroid extract in cancer patients has been discouraged, because it was feared lest the calcium mobilization brought about by this endocrine factor might represent a circumstance favoring the formation of skeletal metastases.[42] As to the manner of action of the parathyroid hormone, it may be pointed out that no difference was found in the increase of the blood calcium level when the hormone was administered to normal or to sarcoma-bearing rabbits.[43]

3. *The Pituitary Gland.*

The complex aspects, exhibited by the pathophysiology of the endocrine secretions of the pituitary gland, are also truly reflected in the studies devoted to the correlations of this organ with neoplasia. Additional obstacles on the road to a clear understanding of the pituitary involvement in these diseases are represented by the fact that extirpation, or implantation, of the whole gland naturally cannot offer a suitable approach for the analysis of the numerous individual hormones produced in this organ, and that pure preparations of these factors, as far as available, frequently do not deserve this designation, and sometimes it is even doubtful whether certain specific effects are actually due to the existence of a separate hormonal agent. Pituitary physiology, originally based on the anatomical division of the gland into an anterior, an intermediate and a posterior lobe, has now arrived at the stage of enumeration of up to ten different hormonal factors, some of which, however, still are subject to dispute as to their actual existence, or as to the anatomical location of their formation. In the following, those studies are first discussed which deal with the organ as a whole; then the survey extends to material on the better defined products of pituitary internal secretions in accordance with the actual progress of general endocrinology.

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[37] A. Inutsuka: Tr. Soc. path. jap. *23*: 680 (1933); Gann *28*: 161 (1934).

[38] R. Reding & A. Slosse: Compt. rend. Soc. de biol. *97*; 1812 (1928); Ann. et bull. Soc. roy. d. sc. méd. et nat. de Bruxelles nos. 9–10, p. 231 (1929).

[39] A. Goerner & B. G. P. Shafiroff: J. Cancer Res. *12*: 294 (1928).

[40] T. S. Paik: Tr. Jap. Path. Soc. *20*: 653 (1930); Am. J. Cancer *15*: 2756 (1931).

[41] F. A. McJunkin, R. D. Templeton & F. G. Kravec: Proc. Soc. Exper. Biol. & Med. *34*: 801 (1936).

[42] E. E. Downs, H. Wammock & R. T. Artman: Am. J. Roentgenol. *37*: 790 (1937).

[43] S. E. Rhee & C. H. Kim: Tr. Jap. Path. Soc. *22*: 974 (1932).

Morphological findings. Several workers believed that the pituitary glands of patients, deceased from neoplasia, or the glands of tumor-bearing animals, histologically exhibited definite deviations from the normal, but no agreement has been reached as to the form of this alteration, or whether it is specific for cancer. Some statements have been forthcoming, reporting on an increase of the principal cells and a decrease of the acidophil cells in the pituitary of cancer patients,[44-46] that is a finding corresponding to that in pregnancy, whereas another author believed he had evidence from his morpho-

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44. Karlefors: Ztschr. f. Krebsforsch. *17*: 195 (1920).
45. W. Berblinger & K. Muth: Zentralbl. f. Gynäk. *47*: 1713 (1923).
46. Y. Ishimoto: Tr. Soc. path. jap. *25*: 654 (1935).

logic studies that in cancer patients an over-function of the anterior pituitary was contrasted by an under-function of the posterior lobe.[47] Therapeutic means derived as a consequence of this assumption will be discussed later on. However, several studies of more recent date resulted in much less specific observations. Although a marked enlargement of the pituitary, involving mainly the anterior lobe, has been found in eighty investigated cancer cases, in contrast to the statements mentioned previously, an increase of acidophil cells became apparent in histological examinations,[48] and another author also noted a supernormal weight of the pituitary in fifty-two cases of malignant tumors, in which histologically a hyperplasia of acidophil cells occurred four times, of chromophobe cells eleven times, and of both chromophobe and of acidophil cells, four times.[49] In particular, cancer of the female genital tract has been reported associated with a marked increase of chromophobe cells, and has been recognized as being definitely distinguished from the aspect of the pituitary in castration.[50] Examination of the pituitary gland of tumor-bearing animals also did not lead to uniform results. In the gland of rats inoculated with Flexner-Jobling carcinoma, findings were observed, reminiscent of the castration effect, viz., an enlargement of the gland and an acidophilia.[51] Analogous findings were obtained on rats with Walker carcinoma, but these were at the same time recognized as being probably of unspecific genesis, since similar changes in the pituitary also followed implantations of kidney tissue, and therefore might be caused merely as a consequence of tissue breakdown and resorption of necrotic masses.[52, 53] However, a later study, in which a review of the earlier papers was presented, stated that in the pituitary of rats with spontaneous, transplanted, or chemically induced neoplasms a decrease of the acidophil, and an increase of the chromophobe cells took place regularly. The latter cells were augmented to more than seventy per cent of their normal amount, and such a change could also be observed in tumor-free animals which had been treated with estrogenic hormones.[54] Hence the alterations of the hypophysial morphology appear to be connected with the endocrine function of the ovary, an aspect which will be discussed later. On the other hand, it was pointed out that the increase of the chromophobe cells and the decrease of the acidophil cells, observed in the pituitary of tumor-bearing rats, is comparable to a similar phenomenon occurring in the glands of tumor-free rats after they have reached a higher age (over eighteen months),[55] and hence the aforementioned histologic findings in cancer patients should be evaluated more closely, with reference to the age of the tested cases. In conflict with the report on an enlargement of the pituitary gland in tumor-bearing animals,[51-53] other investigators found that the weight of the gland in relation to the total brain weight was appreciably reduced (18–23 per cent) in rats with Jensen sarcoma, and it was supposed that this might be connected with the spleen hypertrophy (plus forty-five per cent) occurring in these animals,[56] since the opposite interference—splenectomy

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[47] W. Susman: Brit. M. J. *2*: 794 (1931).

[48] G. A. Wyeth: Endocrinology *18*: 59 (1934).

[49] F. Guccione: Rass. stud. psichiatr. *23*: 507 (1934); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 255 (1935).

[50] B. Zondek: Hormone des Ovariums und des Hypophysenvorderlappens, ed. 2nd, Springer, Vienna 1935, p. 396.

[51] M. F. Guyer & P. E. Claus: Anat. Rec. *52*: 225 (1932); *56*: 373 (1933).

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[52] C. S. McEuen, H. Selye & D. L. Thomson: Brit. J. Exper. Path. *15*: 221 (1934).

[53] C. S. McEuen & H. Selye: Am. J. M. Sc. *189*: 423 (1935).

[54] J. Heiman: Am. J. Cancer *33*: 423 (1938).

[55] J. M. Wolfe, W. R. Bryan & A. W. Wright: Am. J. Cancer *34*: 352 (1938).

[56] J. M. Twort & M. Lasnitzki: Endocrinology *23*: 87 (1938).

—had been previously demonstrated as causing an enlargement of the pituitary gland.[57]

Anterior pituitary hormones in neoplasia. It may be expected that a clearer insight into the interrelationship between the pituitary gland and tumor pathology might be gained by determinations of the internal secretions of this organ. However, at present more detailed information in this respect is available only in regard to the gonadotropic factors of the anterior pituitary, and credit for the initiation of this work must be given to the fundamental studies of Aschheim and Zondek[58] which have been devoted to the endocrinology of the female sex function. In the course of these investigations, it was at first observed that the urinary excretion of gonadotropic substances in a great number of cancer patients deviated from the normal relations, namely in the sense of an increased excretion of follicle-stimulating factors, designated by the authors as prolan A, and contrasted by them with another factor, prolan B, which they believed to be responsible for the luteinization effect of pregnancy urines. Zondek[59] pointed to the dependence of the hormonal excretion on the tumor localization, especially in relation to the genital tract. Gonadotropic substances could be detected in the urine of eighty-two per cent of females afflicted with cancers of the genital organs, whereas they were present in the urines of only thirty-six per cent of female patients suffering from extragenital tumors. In males, testis tumors were almost regularly associated with excretion of large amounts of gonadotropic factors, but in other neoplastic conditions only thirteen per cent of the cases exhibited this hormone in the urine. In later work, the author was able to prove the urinary excretion of luteinizing factors in a number of extragenital cancer cases,[60] a finding which was also obtained by other investigators.[61] In particular, it was reported that positive Friedman's tests, indicating the presence of the luteinizing factor, have been obtained with the urines of five cows suffering from cancers in the eye region,[62] but it is not at present possible to decide whether these findings depended on the endocrine characteristics of this animal species, or were connected with the type of tumors tested. In this connection, reference must be made to the concept of a great number of authors who in contrast to Zondek do not accept a qualitative difference between the follicle-stimulating factor and the luteinizing factor, but they view the urinary gonadotropic principle as a single entity for the different effects of which quantitative relations only are responsible. At any rate, the abnormal urinary excretion of gonadotropic substances in cancer patients has been observed by several authors,[63, 64] some of whom compared this phenomenon to the similar relations occurring in the menopause and, therefore, saw in it the expression of a sexual hypofunction.[65, 66] Such an assumption would make it understandable as to why some studies failed to demonstrate the presence of gonadotropic factors in some, or even in the majority of analyzed urines of patients with tumors of the female genital tract.[67, 68] On the other hand, one author, who found a considerable excretion of gonadotropic substances in the great majority of such cases, believed that this deviation ran parallel to the degree of tumor necrosis and tissue decomposition,[69] and also saw similar relations in a few cases of extragenital tumors of males.[70] In another investigation, the gonadotropic excretion of cancer patients has been determined prior to, and after, radical surgery, and a considerable increase was found following the tumor removal. This was observed especially in breast cancer cases and, the author supposed, indicated a retention of the hormone by the tumors.[71] Support for such a view was seen in the following findings: while injected gonadotropic hormone was retained by cancer-free, and also by cancer-affected women, whose sexual cycle was normal, an excretion of gonadotropic factor following injection of the hormone was observed in cancer-free women without sexual periods, but cancer patients whose cycle had ceased showed a retention of injected hormone.[72] While these results, though very interesting, obviously do not lend themselves for diagnostic purposes, such a possibility seems to exist in regard to the chorionepithelioma of the testis, since a great many studies confirmed the constancy of a urinary excretion of large amounts of gonadotropic substances in these tumors. It became apparent that the quantitative relations were proportional to the tumor advancement, by which means formation of metastases or recurrences may be recognized at an early time.[73-83] The question as to the origin of the hor-

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[57] L. F. Edwards & C. W. Wright: Endocrinology *21*: 808 (1937).

[58] S. Aschheim & B. Zondek: Hormone des Ovariums, Springer, Berlin 1932.

[59] B. Zondek: Klin. Wchnschr. *9*: 679 (1930); Chirurg *2*: 1072 (1930).

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[60] B. Zondek: Hormone des Ovariums und des Hypophysenvorderlappens, Springer, Vienna 1935, pp. 210, 444, 448.

[61] F. Laquer, K. Döttl & H. Friedrich: Medizin und Chemie *2*: 117 (1934).

[62] J. Valasquez & P. Engel: Endocrinology *27*: 523 (1940).

[63] D. Engel: Med. Klin. *26*: 1790 (1930).

[64] Ç. De Fermo: Arch. ital. chir. *33*: 801 (1933); abstr. Ztschr. f. Krebsforsch. *40*: Ref. 96 (1934).

[65] W. Saphir: Endocrinology *18*: 191 (1934).

[66] O. Busse & Klehmet: Zentralbl. f. Gynäk. *59*: 7 (1935).

[67] E. Stöckl: Ztschr. f. Krebsforsch. *41*: 292 (1935).

[68] R. Bolaffi: Riv. ital. ginec. *17*: 191 (1934); abstr. Ztschr. f. Krebsforsch. *42*: Ref. 24 (1935).

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69. U. Baudler: Arch. f. Gynäk. *159*: 101 (1935).
70. U. Baudler: Monatschr. f. Geburtsh. u. Gynäk. *102*: 156 (1936).
71. K. Katz: Beitr. z. klin. Chir. *168*: 448 (1938).
72. K. Katz: Beitr. z. klin. Chir. *170*: 380 (1939).
73. R. S. Ferguson: Am. J. Roentgenol. *29*: 443 (1933); Am. J. Cancer *18*: 269 (1933).
74. A. P. Vastola: J. A. M. A. *101*: 111 (1930).
75. R. B. Henline: J. Urol. *32*: 177 (1934).
76. J. P. Robertson & J. B. Gilbert: J. Urol. *32*: 291 (1934).
77. A. Béclère: Presse méd. *42*: 1513 (1934).

mones, found in the urines of patients with malignant tumors of the testis, has been examined in a recent study. In confirmation of earlier findings,[80] it was demonstrated that it is chorionic gonadotropins which are principally excreted by these patients. In some cases, a simultaneous or successive excretion of hypophysial follicle-stimulating hormone was observed, a phenomenon indicating a sexual hypofunction of the patients concerned.[83a] However, much less light could be shed on the source of the urinary gonadotropic substances in other neoplastic conditions. In this respect, it seemed rather puzzling that hormonal analysis of the pituitary glands of patients with cancer of the cervix showed their content of gonadotropic hormones to be diminished to approximately one-fourth of the normal values.[84] Although normal hormone amounts were found in the pituitaries of some other cancer types, an increase of these factors could never be detected, not even in patients suffering from very malignant tumors.[85] The glands of rats with Jensen sarcomas also exhibited a slightly reduced content of gonadotropic factors.[86] An alternative possibility, namely, the production of these hormones in the tumors themselves, does not seem very probable, since pertinent analyses of human neoplasms,[87] or of Jensen sarcomas,[86] led to negative results, and only one report is avail-

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78. C. C. Herger & A. A. Thibaudeau: Am. J. Cancer *22*: 525 (1934).

79. A. G. Chiariello: Ann. ital. chir. *13*: 774 (1934); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 256 (1935).

80. H. M. Evans & P. R. Austin: J. Exper. Med. *58*: 561 (1933).

81. M. Cutler & S. E. Owen: Am. J. Cancer *24*: 318 (1935).

82. H. C. Fortner & S. E. Owen: Am. J. Cancer *25*: 89 (1935).

83. Ch. Hamburger, F. Bang & J. Nielsen: Acta path. et microbiol. Scandinav. *13*: 75 (1936).

83a. Ch. Hamburger: Acta path. et microbiol. Scandinav. *18*: 457 (1941).

84. B. Zondek: loc. cit. 60, p. 179.

85. B. Zondek: J. A. M. A. *108*: 607 (1937).

86. H. Druckrey: Arch. f. exper. Path. u. Pharmakol. *180*: 367 (1936).

87. M. Borst, A. Döderlein & D. Gostimirović: München. med. Wchnschr. *78*: 2108 (1931).

able according to which gonadotropic substances have been isolated from mammary tumors of mice.[88] It must, however, be stressed that the increased urinary excretion of gonadotropic substances, as far as observed in cancer patients, does not necessarily represent a consequence of augmented hormone production, but the urinary hormone output might be connected with other deviations of the hormonal metabolism, e.g., the ability of the organism to retain the hormone, or a change of the renal threshold for its excretion. On the other hand, the finding of low hormone concentrations in the hypophysis does not necessarily exclude the possibility of an increased secretion, since the hormone might disappear rapidly from the organ after its production.

To the knowledge of the authors, analyses relating to the secretion of other hormones of the anterior pituitary lobe in neoplasia have not been recorded, with the exception of a series of studies performed within the last years and dealing with a new hormonal factor, which was found capable of stimulating the reticulo-endothelial function, as indicated by the removal of Congo red from the blood stream. This "restropic" factor was found to be lacking in the blood of cancer patients, or tumor animals, in contrast to the blood of tumor-free humans or animals,[89] and its gradual disappearance was observed in the blood of rabbits treated with carcinogenic hydrocarbons.[90] A more thorough discussion of these phenomena, bearing on the problem of the involvement of the reticulo-endothelial system in neoplasia, will be given in chapter VIII. Some investigations may be mentioned here, the subject of which has been antihormone factors found to exist in the organisms of animals or humans affected with cancer. In this respect it was noted that injections of gonadotropic substances into mice failed to elicit the characteristic response of the ovary (Aschheim-Zondek reaction), or in the vagina (Allen-Doisy reaction), after the animals had been inoculated with Ehrlich sarcoma,[91] and the presence of antigonadotropic factors, i.e., of substances which counteract the known effects of gonadotropic hormones, has been detected in about eighty per cent of the urines of cancer patients.[92] A diagnostic utilization of this phenomenon has been considered, but the studies on this subject do not seem to have advanced far enough to warrant final conclusions. The same holds true for an investigation, in which an antigonadotropic quality has been ascribed to the serum of cancer patients, since the experimental material was very small.[93] The author of the latter study believed that antihormonal factors generally were increased in neoplasia and, in this connection, reference is made to a finding mentioned above,[7] according to which a principle directed against another hypophysial secretion, viz., an antithyrotropic factor, was found in seventy-five per cent of cancer serum. Studies devoted to factors cancelling the effect of the chromatophorotropic hormone, a product of the intermediate pituitary lobe, will be discussed later.

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[88] N. Dobrovolskaia-Zavadskaia & P. Zéphiroff: Compt. rend., Acad. d. sc. *198*: 1950 (1934).

[89] C. Wetzler-Ligeti & B. P. Wiesner: Nature, London *140*: 892 (1937); *141*: 1100 (1938); Endocrinology *22*: 693 (1938).

[90] T. Csato, C. Wetzler-Ligeti & B. P. Wiesner: Am. J. Cancer *36*: 257 (1939).

Pituitary gland and tumor growth. One approach, which has been frequently used for determining the influence of the pituitary gland on the neoplastic process, has been the total hypophysectomy, and several experiments have been recorded which agreed that the growth of animal tumors was impeded when the inoculation was performed following[94–96] or prior[97–100] to the removal of the gland. In one of the studies, an inhibition of the formation of metastases was noted,[97] and an effect, similar to the surgical extirpation, could also be achieved by roentgen irradiation of the gland.[98, 99] In addition to these results obtained with transplanted tumors, analogous relations have been demonstrated in regard to chemically induced tumors, inasmuch as hypophysectomy was seen to impede the development of neoplasms in rats treated with dibenzanthracene,[101] or in rabbits which were painted with benzpyrene,[102] although in the latter instance it must be kept in mind that this animal species in general has been found to be not very susceptible to this particular carcinogenic stimulus (cf. chapter II). In interpreting the aforementioned findings, there can be no doubt that the procedure of hypophysectomy must be considered as causing a very far-reaching interference, the effects of which are certainly not confined to the exclusion of the pituitary hormones, but also indirectly affect many other endocrine glands and the general health of the animals. Nevertheless, some authors communicated observations which should support their view that a specific influence of the hypophysectomy on the neoplastic process exists, inasmuch as the subsequent growth inhibition of tumors could be cancelled by injections of pituitary extracts,[98] or since a growth inhibition of the

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[91] J. Flaks & A. Ber: Bull. Assoc. franç. p. l'étude du cancer *27*: 193 (1938).

[92] J. Flaks & A. Ber: Compt. rend. Soc. de biol. *130*: 306 (1939); Bull. Assoc. franç. p. l'étude du cancer *28*: 337 (1939).

[93] W. Rodewald: Klin. Wchnschr. *18*: 26 (1939).

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94. C. S. McEuen: Proc. Soc. Exper. Biol. & Med. *30*: 928 (1933).
95. H. A. Ball & L. T. Samuels: Am. J. Cancer *16*: 351 (1932).
96. L. T. Samuels & H. A. Ball: Am. J. Cancer *18*: 380 (1933).
97. C. S. McEuen & D. L. Thomson: Brit. J. Exper. Path. *14*: 384 (1933).
98. F. Bischoff, L. C. Maxwell & H. J. Ullman: Am. J. Cancer *21*: 329 (1934).
99. L. T. Samuels & H. A. Ball: Am. J. Cancer *23*: 801 (1935).
100. K. Narimatsu: Jap J. Obst. & Gynec. *20*: 397 (1937).
101. H. A. Ball & L. T. Samuels: Am. J. Cancer *26*: 547 (1936).
102. A. Lacassagne & W. Nyka: Compt. rend. Soc. de biol. *121*: 822 (1936).

Walker carcinoma has been found in hypophysectomized rats, in spite of the fact that in consequence of stomach tube feeding, the relations of body weight and caloric intake in the treated animals equaled the relations existing in control animals.[103] An indication for the alteration of metabolic processes, caused by the extirpation of the pituitary gland, may be inferred from the finding of an increased glycogen content in the more slowly growing Walker tumors 256 of hypophysectomized rats, as compared with tumors of control animals, when both groups were fed a glycogen-rich diet ([104], cf. chapter II). But, on the other hand, reference must be made to several studies which yielded results of much less impressive nature concerning the influence of hypophysectomy on the tumor process. Only a moderate growth inhibition was observed in transplanted tumors of hypophysectomized mice, and in analogously treated animals the appearance of benzpyrene tumors was found only to be delayed, but not inhibited.[105] Likewise, hypophysectomy of rats exerted only a slight growth inhibition on inoculated tumors.[106] Completely negative results were obtained in experiments in which the pituitary gland of rabbits had been destroyed by implantation of radium seeds, since Brown-Pearce carcinomas inoculated six weeks prior to this treatment, or shortly after it, did not seem affected by this interference.[107] Furthermore, when Walker carcinomas were transplanted to rats, three to four weeks following a hypophysectomy, no alteration of their metabolism in vitro could be demonstrated when compared with tumors of control animals.[108] An interesting finding has been reported as to the influence of hypophysectomy on Kato rabbit sarcomas. A stimulation of the tumor growth took place in the first period after the removal of the gland, but was later replaced by an inhibition.[109] It may be deduced from this, and the previously mentioned findings, that the effects of hypophysectomy on tumor growth depend in their nature and extent on disturbances of the hormone equilibrium, which are caused by the removal of this gland which influences so many other endocrine organs. This interference may, under certain not fully clarified conditions, antagonize the neoplastic process, but it cannot be considered as a means of specifically counteracting neoplasia. Similar complex relations may be expected to be involved in the few recorded investigations in which implantations of hypophysis were performed. In one of these studies, it was found that implantation of rat pituitary glands to rats depressed the takes of inoculated carcinomas by fifty per cent, whereas implantation of pituitary glands of pigs were ineffective.[110] However, other authors reported that the implantation of anterior hypophysis into female mice of a pure strain significantly increased the incidence of mammary cancer and, since this effect did not take place in castrated mice, it was assumed that the tumor promoting influence depended on a stimulation of the ovarian secretion,[111] a problem which there will be occasion to discuss later on. At any rate, it seems obvious that the action of pituitary implants must be understood in the light of the endocrine factors which were thus introduced into the body, and which may in turn act upon other glands with internal secretion.

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[103] H. A. Ball & L. T. Samuels: Am. J. Cancer *32*: 50 (1938).

[104] H. F. Schott, L. T. Samuels & H. A. Ball: Proc. Soc. Exper. Biol. & Med. *37*: 410 (1937).

[105] R. Korteweg & F. Thomas: Am. J. Cancer *37*: 36 (1939).

[106] L. A. Emge & K. M. Murphy: Am. J. Obst. & Gynec. *32*: 593 (1936).

[107] A. Lacassagne & W. Nyka; Compt. rend. Soc. de biol. *122*: 747 (1938).

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108. C. C. Franseen & C. McTiernan: Am. J. Cancer *26*: 106 (1936).
109. Y. Nitta: Jap. J. Obst. & Gynec. *20*: 368 (1937).
110. R. E. Gardner: Am. J. Hyg. *23*: 415 (1936).
111. L. Loeb & M. M. Kirtz: Am. J. Cancer *36*: 56 (1939).

This leads to the great number of investigations in which pituitary extracts have been administered for the purpose of affecting the cancerous process, although here also uniform results have not been reported. This is by no means surprising, as far as experiments are concerned in which crude pituitary extracts were employed, since they contained more than one endocrine factor and hence were probably of varying qualitative and quantitative composition. In animal experiments, anterior pituitary extracts seemed to stimulate the growth of inoculated tumors,[112, 18] and while some authors stated they had achieved therapeutic successes in inoperable cancer patients by means of injections of preparations obtained from the anterior hypophysial lobe,[113, 114] conflicting findings exist which obviously indicate the prevalence of different active factors present in the preparations employed. In this respect, it has been made very probable that the anterior hypophysis secretes two groups of hormones, which are antagonistic as to their effect on tumor growth. These are Evan's growth hormone, on the one hand, and the gonadotropic factors, on the other. The first seems to stimulate neoplastic growth, and its presence was most likely responsible for the increased growth of animal tumors observed following the administration of anterior pituitary extracts,[115–118] an assumption which is in agreement with its established ability to promote normal growth. Furthermore, analogous results—stimulation of tumor growth—have been

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112. D. Engel: Ztschr. f. Krebsforsch. *19*: 339 (1923).
113. R. H. Norgate: Brit. M. J. *2*: 280 (1920).
114. E. Vanýsek: Časop. lék. česk. 1936: 1321; abstr. Ztschr. f. Krebsforsch. *45*: Ref. 347 (1937).
115. T. B. Robertson & T. C. Burnett: J. Exper. Med. *21*: 280 (1915).
116. T. Hayashi: Tr. Jap. Path. Soc. *20*: 661 (1930).
117. F. Bischoff, L. C. Maxwell & H. J. Ullmann: Proc. Am. Soc. Biol. Chem., 26th meet., p. 102 (1932).
118. K. Sugiura & S. R. Benedict: Am. J. Cancer *18*: 583 (1933).

obtained by using pure preparations of the pituitary growth hormone.[67, 119-122] But at the same time it must be stressed that these findings cannot be viewed as proving the indispensability, or even an etiologic significance, of this hypophysial hormone for tumor development. That this is not the case has been clearly shown in experiments in which pituitary dwarfism has been produced in rats by means of protracted injections of estrogens, a treatment which caused a definite damage of the secretion of the growth hormone; since in these animals benzpyrene administration resulted in tumor formation in the same manner as in untreated controls, it seems established that carcinogenesis may also take place in an organism, in which the production of this hormone is impaired or lacking.[123] Moreover, some investigators failed to notice any influence of Evan's hormone on rat sarcomas growing in normal or hypophysectomized animals.[106] Hence the stimulation of tumor growth, seen in the studies mentioned above after the administration of the growth hormone, is not to be regarded as a constant phenomenon and, most likely, merely expresses a response of neoplasms, which is analogous to the activation of normal growth due to this hormone.

The opposite effect, i.e., an inhibition of tumor growth, has been ascribed to gonadotropic factors, the administration of which markedly retarded the advancement of Ehrlich mouse carcinomas, although it never resulted in complete tumor regression; the weight of tumors of treated animals averaged one-eighth of that of control tumors and the virulence of the treated neoplasms appeared definitely diminished,

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119. P. Engel: Ztschr. f. Krebsforsch. *41*: 281 (1935).
120. H. Druckrey: Deutsche med. Wchnschr. *62*: 717 (1936).
121. K. Narimatsu: Jap. J. Obst. & Gynec. *20*: 391 (1937).
122. F. Bischoff & M. L. Long: Am. J. Cancer *31*: 67 (1937).
123. B. Zondek: Lancet *1*: 689 (1937).

as evidenced by their failure to grow when inoculated into other animals. Some benefits were also observed from a treatment with high doses of gonadotropic hormones in cancer patients, particularly insofar as a gain of weight took place.[124] Corroborative findings, indicating a growth inhibition of various animal tumors by treatment with gonadotropic substances, have been announced by a number of authors.[67, 119, 125–130] Although true cures could not be achieved, and very large hormone doses had generally to be employed (up to 2,300 rat units), [127] the administration of gonadotropic substances was reported to bring about at least subjective improvements in cancer patients,[131] sometimes causing histological alterations of the tumors.[69] The addition of gonadotropic factors was seen also to impede the growth of human neoplastic tissue cultures, and a diminished yield of tumors was obtained in animals when the inoculated material had been previously treated with this hormone.[132] In contrast to the latter finding, however, another study failed to reveal any difference in the growth of tumors which had been treated with gonadotropic factors prior to their inoculation.[133] This leads us to numerous observations of other authors who reported negative results from their experiments which analyzed the influence of gonadotropic substances on tumor

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124. H. Zondek, B. Zondek & W. Hartoch: Klin. Wchnschr. *11*: 1785 (1932).

125. H. Möller: Frankfurt. Ztschr. f. Path. *45*: 571 (1933).

126. F. Savignoni: Rass. clin. *32*: 349 (1933); abstr. Ztschr. f. Krebsforsch. *40*: Ref. 98 (1934).

127. L. Cannavò: Riforma med. *49*: 278 (1933); abstr. Am. J. Cancer *19*: 405 (1933).

128. B. Lustig & H. Wachtel: Biochem. Ztschr. *271*: 345; 357 (1934).

129. P. Engel: Ztschr. f. Krebsforsch. *41*: 480 (1935).

130. M. A. Magath & E. Smoilowskaja: Vopr. Onkol. *7*: 225 (1935); abstr. Ztschr. f. Krebsforsch. *46*: Ref. 64 (1937).

131. L. Kriesch & K. Vietorisz: Zentralbl. f. Gynäk. *58*: 2370 (1934).

132. F. Ludwig & J. v. Ries: Schweiz. med. Wchnschr. *65*: 5 (1935).

133. S. Tanzer: Am. J. Cancer *26*: 102 (1936).

growth in animals,[110, 134–140] or who found no beneficial effects from an analogous clinical treatment,[141] in spite of the fact that the hormone dosage employed in some of these studies was very high. A circumstance which perhaps is responsible for some of these discrepancies may be the fact that substances of different origin employed in the experiments have been grouped together under the designation "gonadotropic substances," namely the internal secretion of the anterior pituitary on the one hand and, on the other, substances excreted during pregnancy in the urine, the origin of which latter factors is the placenta, and the complete identity of which with the pituitary hormone has been questioned by some authors. But whereas one investigator observed a different effect on animal tumors as resulting from the administration of anterior pituitary factors, or of chorionic gonadotropic hormones,[129] others obtained negative results, both with the urinary and the pituitary factors,[140, 141] or they saw only a temporary inhibition of animal tumors following administration of the follicle-stimulating hormone of the anterior hypophysis.[142] However, the fact has been definitely established that crude preparations of gonadotropic substances, obtained from pregnancy urines, exert a marked inhibitory effect on animal tumors; but this quality appears to be lost in the course of the purification of the sub-

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134. L. Gross: Ztschr. f. Krebsforsch. *38*: 289 (1932).
135. O. M. Gruhzit: Arch. Path. *16*: 303 (1933).
136. O. F. Krehbiel, C. D. Haagensen & H. Plantenga: Am. J. Cancer *21*: 346 (1934).
137. B. P. Wiesner & A. Haddow: Nature, London *132*: 97 (1933).
138. C. S. McEuen & D. L. Thomson: Brit. J. Exper. Path. *15*: 224 (1934).
139. I. P. Miscenko & E. P. Gossdikovskaja: Vopr. Onkol. *7*: 211 (1935); abstr. Ztschr. f. Krebsforsch. *46*: Ref. 64 (1937).
140. F. Bischoff & L. C. Maxwell: Am. J. Cancer *27*: 87 (1936).
141. G. Ernst: Strahlentherapie *48*: 552 (1933).
142. Y. Nitta: Jap. J. Obst. & Gynec. *19*: 520 (1936).

stances.[143, 120] While highly purified urinary hormones were found completely ineffective, a fraction of the urine, which was indialysable and soluble in eighty per cent ethyl alcohol, was found to retard somatic and tumor growth.[144] Thus it appears likely that some of the effects ascribed to gonadotropic substances have actually been due to impurities of the hormone preparations used, especially as far as they were derived from pregnancy urines. This circumstance possibly may serve as an explanation for conflicting statements which have been recorded as to the influence of gonadotropic substances on tumor development. Some authors did not see any delaying effect on the tar cancer formation from the administration of hormones of the anterior pituitary,[145, 146] or they even noted an increased yield of tumors [147] or enhancement of the cancer development in mice painted with tar.[148, 149] But in one study it was reported that injections of chorionic gonadotropic factor strongly inhibited the tar tumor development,[150] and the production of hepatomas in rats by means of feeding aminoazotoluene was claimed to be impeded by a similar treatment.[151] Only few studies employed spontaneous animal tumors, and in these cases injections of gon-

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143. F. Bischoff & L. C. Maxwell: J. Pharmacol. & Exper. Ther. *52*: 378 (1934).

144. F. Bischoff, L. C. Maxwell & M. L. Long: Am. J. Cancer *31*: 72 (1937).

145. K. Sciesiński: Bull. internat. acad. pol. sc.; cl. méd. 1931: 225; abstr. Ztschr. f. Krebsforsch. *37*: Ref. 72 (1932).

146. H. W. Julius: Acta brev. neerl. physiol. *4*: 74 (1935); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 103 (1935).

147. E. Maurizio & E. Debiasi: Monit. ost. e ginec. *5*: 321 (1933); abstr. Ztschr. f. Krebsforsch. *40*: Ref. 179 (1934).

148. J. Zeldenrust: Acta brev. neerl. physiol. *4*: 182 (1935); abstr. Ztschr. f. Krebsforsch. *42*: Ref. 78 (1935).

149. I. K. Issakowa, E. A. Pretschistenskaja & S. S. Rogowenko: Akuš. i ginek. no. 11: 1358 (1936); abstr. Ztschr. f. Krebsforsch. *46*: Ref. 64 (1936).

150. K. Katz: Ztschr. f. Krebsforsch. *45*: 139 (1937).

151. S. Ito: Gann *34*: 31 (1940).

adotropic substances retarded the onset and decreased the total incidence of mammary adenocarcinoma in virgin female mice of the Marsh-Buffalo strain,[152] whereas the growth of already developed spontaneous mouse tumors was not found impeded by this treatment.[153] An entirely different kind of action is apparently involved in the phenomenon, in that the production of testicular teratomas in fowl, by means of local injections of zinc chloride, could be appreciably enhanced. They could be induced independently of the time of the year when the animals received injections of gonadotropic hormones, whereas untreated animals responded to zinc chloride with tumor formation only during the first three months of the year.[154] As was described in detail in the first chapter, this carcinogenic mechanism was recognized as being caused by an excessive regeneration of the damaged testicular tissue, and as requiring endocrine stimulation, which may be provided by the ingestion of gonadotropic substances. It is very probable that the well known stimulating effect of the anterior pituitary was likewise responsible for the reported finding of an increased epithelial proliferation of the uterine cervix in guinea pigs which had been treated with injections of anterior pituitary hormones,[155] but it appears very doubtful whether any conclusions as to an etiological significance of these hypophysial hormones for the formation of genital tumors may be derived from this observation.[156] A similar interpretation seems to be applicable to the results obtained by another author who administered injections of gonadotropic substances, prepared from pregnancy urines, to sixteen female rabbits during one year. The following observations were reported; in all animals atrophy of the uterine mucosa—particularly of the glands—occurred, in some of the animals inflammatory reactions set in, and in two of them adenomas developed which penetrated the muscular layer.[157] To the writers' belief, these findings cannot be considered as proof for a true tumorigenic quality of the gonadotropic factors, but they should rather be viewed as the consequence of a severe disturbance of the endocrine balance, due to the prolonged administration of these hormones. Another viewpoint, regarding the theoretical basis for the influence of anterior pituitary hormones on the neoplastic process, has been advanced, inasmuch as the effect of the endocrine factors on the tumor metabolism has been considered as being of decisive importance. In this connection, it was assumed that an antagonism existed between the action of the growth hormone and the action of the gonadotropic factors, of which the first should increase the oxygen consumption of tumor cells, whereas the latter should lower it and hence increase the formation of lactic acid in tumors.[158] Since it was further believed that such an extreme increase of glycolysis would damage the vitality of neoplastic cells, on account of the excess accumulation of lactic acid, therapeutic recommendations have been based on these relations,[159] which are discussed in chapter VI. It must, however, be remarked that the glycolysis of uterine cancer in vitro was found to be decreased by thirty six per cent when anterior pituitary extract was added,[11] and that the glycolysis of animal tumors has not been found to be affected when gonadotropic substances were injected in vivo.[160]

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[152] F. Bischoff, M. L. Long, J. J. Rupp & G. J. Clarke: Endocrinology *28*: 769 (1941).

[153] H. Baatz: Ztschr. f. Geburtsh. u. Gynäk. *120*: 70 (1939).

[154] H. J. Bagg: Am. J. Cancer *26*: 69 (1936).

[155] J. Hofbauer: Klin. Wchnschr. *9*: 2108; 2153 (1930).

[156] H. Hartmann & H. Olbers: Zentralbl. f. Gynäk. *55*: 1314 (1931).

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[157] H. Pierson: Ztschr. f. Krebsforsch. *45*: 1 (1937).

[158] M. Reiss & A. Hochwald: Med. Klin. *28*: 1391; 1432 (1932).

[159] M. Reiss: 1st Internat. Cancer Congress, Madrid *2*: 410 (1933); Med. Klin. *28*: 1100 (1932).

[160] W. Heinrichs: Thesis, Königsberg i. Pr. (1933); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 21 (1935).

Only few investigations have dealt with other internal secretions of the anterior hypophysis, among which a particular significance may be assigned to some interesting observations regarding the thyrotropic hormone in connection with the development of spontaneous mouse tumors. When animals of an inbred strain with high mammary cancer incidence received injections of this hormone, they were found to remain tumor-free at an age where in fifty per cent of the control animals tumors had arisen.[161] Although some reexaminations of this finding failed to confirm it,[162, 162a] the author of the first communication sustained his original statement on the basis of his own experimental evidence, and he explained the opposing results as being due to a different interpretation of the statistics, to differences in the strains employed and in the breeding activity of the respective animals, and perhaps as being due to an insufficient dosage of the hormone.[163] The author emphasized, it is worth noting, that he did not regard the thyrotropic hormone as having cancer curative properties, but merely as being capable of restoring a hormonal equilibrium which, he assumed, had been disturbed in the animals susceptible to spontaneous tumor development. Comparatively large doses of prolactin, a product of the anterior pituitary lobe, which stimulates the secretion of the lactating breast, were found to be without any effect on transplanted rat sarcomas.[140]

A disturbance of the fat metabolism has been detected in cancer patients, inasmuch as fat ingestion did not cause an increase of the acetone bodies in their blood, a phenomenon which occurs in normal persons under these conditions, and it was reported that the administration of an anterior pituitary extract restored this increase of the acetone bodies to normal proportions.[164] This finding might indicate a deviation of the fat metabolism in neoplasia, due to a defective function of the hypophysis, but its exact evaluation must await further investigations.

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[161] W. Cramer & E. S. Horning: Lancet *1*: *72* (1938).

[162] C. D. Haagensen, H. T. Randall & R. Auchincloss: Proc. Soc. Exper. Biol. & Med. *45*: 820 (1940).

[163] W. Cramer: Am. J. Cancer *40*: 431 (1940).

The intermediate lobe of the hypophysis. The investigations devoted to this part of the hypophysis have been exclusively concerned with the chromatophorotropic hormone (intermedin), so far the only endocrine factor known to be produced in this organ. It must be anticipated that the interpretation of the results obtained will encounter essential difficulties for two reasons. Firstly, the physiological significance of this hormone in mammals is still obscure and, secondly, the tests for its standardization, based on its property of causing expansion of pigment cells in certain fish and amphibia, may yield unspecific, misleading results when precautionary measures, such as careful selection of the test animals (the fish species phoxinus has been found to represent the most suitable subject) or accurate neutralization of the injected material are neglected, and these factors may be responsible for conflicting statements mentioned in the following. The concentration of intermedin in the pituitaries of persons deceased from cancer has been found to reach very high values (10,000 to 13,000 phoxinus units) only in far advanced liver cancer, but this alteration did not appear generally in neoplasia, and, furthermore, high hormone values have also been determined in the glands of patients who died of other diseases, e.g., uremia.[165] Therefore, the findings of another author, implying a generally decreased intermedin content in the hypophyses of tumor patients,[166] are in need of further re-examinations in order to sustain their validity. While no active hormone can be isolated from the blood of normal persons, it has been reported that it was detected in the blood of one patient suffering from melanosarcoma, but the hormone was apparently not excreted in the urine.[167] In another study it was claimed that positive melanophore reactions in frogs were obtained when the animals received injections of urines of patients suffering from cancers of the female genital tract, and a diagnostic utilization of this finding was considered, since the urines of normal persons, or of patients not afflicted with malignancy, did not contain the hormone.[168] Other authors, however, who obtained positive melanophore reactions in albino salamanders with forty-one urines of cancer patients, stated that a similar response was obtained when urines of pregnant women, or of patients with Graves' disease, or pulmonary gangrene, etc., were injected into the animals,[169] thus making a diagnostic usefulness of this phenomenon very doubtful. A great number of studies have been performed by one investigator with regard to a phenomenon, detected by her, namely an inactivation of melanophore hormone in vitro, when sera of cancer patients were added.[170] Although similar experiments with sera of pregnant women or of patients suffering from some nonmalignant diseases likewise indicated an inactivation of the hormone, a diagnostic method has been developed on the basis of this observation which, it was claimed, yielded only seven per cent of erroneous results. However, a re-examination, in which a very sensitive method (hypophysectomized frogs) was used, was not able to confirm the presence in cancer serum of factors which inactivate the effect of melanophore hormone.[171] As to the influence of the hormone of the intermediate pituitary lobe on tumor growth, animal experiments showed a slight stimulation of the growth of inoculated mouse tumors as following repeated injections of intermedin.[172] Such a tumor promoting quality of this hormone has tentatively been taken into consideration, on account of a clinical observation; that is, the complete disappearance of extensive generalized metastases of a malignant melanoma, following intensive roentgen irradiation of the hypophysis of the patient, was observed.[173] Unquestionably, further clinical experiences, as well as laboratory work, are required before any definite conclusions can be arrived at in this connection, but it seems possible that the melanophore hormone may in some way be involved in the pathology of malignant tumors arising from pigment cells.

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[164] Gaessler: Zentralbl. f. Gynäk. *58*: 258 (1934).

[165] B. Zondek: Die Drüsen mit innerer Sekretion, Aeskulap-Verlag, Vienna 1937; p. 104 seq.

[166] W. Rodewald: Ztschr. f. Krebsforsch. *48*: 161 (1938).

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[167] E. B. Astwood & C. F. Geschickter: Am. J. Cancer *27*: 493 (1936).
[168] R. Umezawa: Jap. J. Obst. & Gynec. *18*: 2 (1935).
[169] H. Nakamura, J. Tanaka & Y. Yohda: Gann *34*: 6 (1940).
[170] W. Rodewald: Deutsche med. Wchnschr. *62*: 726 (1936); *63*: 1271; 1362 (1937): Ztschr. f. Krebsforsch. *47*: 540 (1938)

The posterior pituitary. The scientific exploration of this organ in connection with neoplasia has so far yielded only very few definite results. No actual determination of its hormone output in this pathology has been recorded, but, as mentioned above, one author, on the basis of histologic findings, assumed that tumor patients exhibited an insufficiency of the posterior pituitary, and he even believed that a successful cancer therapy may be based on the administration of posterior pituitary extracts to patients kept on a carbohydrate-poor diet.[49] However, a test of this treatment led to negative results as to its beneficial effect on cancer patients [174] and, likewise, animal experimentation with posterior pituitary hormones resulted in contradictory reports. Some investigators found a growth inhibition of transplanted mouse carci-

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171. J. H. Last & E. M. K. Geiling: Am. J. Cancer *38*: 380 (1940).
172. K. Sugiura: Proc. Soc. Exper. Biol. & Med. *32*: 375 (1934).
173. P. E. Wigby & M. H. Metz: Am. J. Roentgenol. *41*: 415 (1939).
174. W. Stewart: Brit. M. J. *1*: 98 (1932).

noma,[175] or of tar cancer of mice,[176] or of the Kato rabbit sarcoma,[177] following the administration of these endocrine factors, but other workers failed to notice any such influence on animal tumors.[178-181] It must be remarked that the experiments so far have not employed isolated hormones of the posterior hypophysis (oxytocin, pitocin), of which latter it was merely stated that their administration in vivo inhibited the glycolysis in rat sarcomas, subsequently tested in vitro.[160] An announcement has been made that a factor, called "amicin," had been obtained from the posterior hypophysis, which exhibited an effect antagonistic to that of Evan's hormone, by inhibiting the growth of plant seedlings, of young animals, and also of transplanted tumors,[182] but more experiences must be collected on this subject before a definite evaluation is possible.

4. *The Pineal Gland.*

The way has only recently been prepared for an investigation of the interrelationship of this organ with tumor pathology, which is not surprising since our knowledge on the normal endocrinology of the pineal gland has not progressed too far, partly on account of technical difficulties encountered in experimental work with this organ. Therefore a very critical attitude must be maintained towards an older com-

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175. G. Hallemann: Vorläufige Mitteilung, Selbstverl., Dresden 1931; abstr. Ztschr. f. Krebsforsch. *37*: Ref. 71 (1932).

176. L. Seel: Ztschr. f. Krebsforsch. *22*: 1 (1925).

177. K. Narimatsu: Jap. J. Obst. & Gynec. *20*: 387 (1937).

178. V. Korentchevsky: Compt. rend. Soc. de biol. *83*: 781; 783 (1920).

179. E. W. Riches & M. Kremer: Brit. M. J. *1*: 877 (1932).

180. S. W. Houston & J. Miller: Tr. roy. Soc. Canada V Biol. Sc. III s. *26*: 71 (1932); abstr. Ztschr. f. Krebsforsch. *39*: Ref. 62 (1933).

181. L. C. Maxwell & F. Bischoff: J. Pharmacol. & Exper. Ther, *49*: 270 (1933).

182. H. Wachtel & B. Lustig: Warszawskie casop. lek. *15*: No. 36/37 (1938).

munication, according to which spontaneous tumors have been cured in mice and transplanted tumors have been retarded in growth by means of epiphysectomy,[183] because it hardly can be believed that the interference—removal of this small organ in mice—did not in fact also produce other damage, and, moreover, the evaluation of these experiments is made difficult by the circumstance that implantation of testicular tissue was performed in the same animals. No confirmations of these results have been forthcoming. On the contrary, indirect evidence of other experiments seems to point to an opposite conclusion, i.e., the administration of pineal extracts was reported markedly to inhibit the growth of inoculated mouse tumors.[119, 184] Mouse carcinomas appeared less susceptible to the treatment than sarcomas, and no prolongation of the life duration was observed, obviously on account of a general toxic effect exerted by the glandular extract. Nevertheless, the author in question declined to see in the tumor inhibition merely an expression of the impaired health condition of the animals treated, but he believed that a true antagonism existed between the action of the anterior pituitary growth factor and the pineal hormone, as to their influence on tumor growth, and he interpreted the effect of the latter as being a "hormonal hypophysectomy." [185] Yet in view of the relations pertaining to the hypophysial hormones, as outlined in the preceding section, and in view of another study, in which administration of pineal extracts had variable effects on the growth of mouse tumors,[186] one does not seem warranted in assuming a definite connection between tumor growth and pineal secretion.

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[183] E. Georgiou: Ztschr. f. Krebsforsch. *28*: 562 (1929).
[184] P. Engel: Ztschr. f. de ges. exper. Med. *93*: 69 (1934).
[185] P. Engel: Wien. klin. Wchnschr. *48*: 481 (1935).
[186] N. Dobrovolskaia-Zavadskaia & P. Zéphiroff: Compt. rend. Soc. de biol. *134*: 60 (1940).

5. *The Adrenal Glands.*

These organs represent an anatomical entity in mammals, but are sharply divided as to their endocrine function into two different parts, the adrenal cortex, and the adrenal medulla. The latter belongs to the chromaffine tissues, parts of which are localized also in other parts of the body, and logically a separate investigation should be applied to these two systems regarding their correlation to the neoplastic process.

Morphological findings. A considerable enlargement of the adrenal glands has been observed in fowl with Rous sarcoma,[187] in rats inoculated with Flexner carcinoma,[188] in rats bearing Walker tumor 256,[189] and in rabbits affected with tar cancer.[190] The hypertrophy mainly concerns the cortex, and further studies stated more specifically that this phenommenon occurred in the first stages of the tumor process, while it is later replaced by degenerative changes.[191, 192] Similar alterations have been obtained after injections of alcoholic extracts of rabbit sarcoma to normal animals,[191] and since a hypertrophy of the adrenal cortex, appearing in rats with Walker tumors, failed to occur in hypophysectomized tumor-bearing animals, it has been supposed that tumor breakdown products exert a stimulating influence on the pituitary production of adrenotropic hormone, which in turn would be responsible for the increased functional activity of the adrenal cortex.[193] An analogous explanation, that is, an unspecific cause due to the liberation of necrotic tumor material, has

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187. B. Sokoloff: Arch. f. exper. Zellforsch. *11*: 112 (1931).
188. T. Tamura: Jap. J. Obst. & Gynec. *17*: 349 (1934).
189. B. Sure, R. M. Theis, R. T. Harrelson & L. Farber: Am. J. Cancer *36*: 252 (1939).
190. M. Oike: Tr. Jap. Path. Soc. *20*: 655 (1930).
191. S. Okamoto: Jap. J. Obst. & Gynec. *18*: 302 (1935).
192. B. Sokoloff & I. Arons: Am. J. Surg. *49*: 471 (1940).
193. H. A. Ball & L. T. Samuels: Proc. Soc. Exper. Biol. & Med. *38*: 441 (1938).

also been applied to the findings of a leucocytic infiltration of the adrenal cortex in tumor-bearing animals,[55, 194] or in humans deceased from malignant tumors.[195] But a far more specific significance has been assigned to the discovery of a brown degeneration, occurring in the reticular zone of the adrenal cortex in mice of inbred strains exhibiting a high incidence of spontaneous mammary cancer; since this phenomenon was observed prior to the tumor development, and was not found in animals of low-tumor strains, the authors assumed that it was connected with the hereditary susceptibility to neoplasia, and considered it as an indication of a disturbed endocrine balance favoring tumor formation. The latter view seemed supported by the fact that similar histologic changes were noted in animals following administration of estrogenic substances,[196] the part of which in tumor pathology will be discussed later. Confirmations of these findings have been presented by several investigators,[197-201] but since others were not able to establish a correlation between the brown degeneration observed in the adrenal cortex of various animal strains and their respective tumor incidence,[202, 203] it appears doubtful whether a general validity can be assigned to the phenomenon in question.

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194. M. R. Lewis: Am. J. Cancer *30*: 95 (1937).
195. A. M. Sala & R. J. Stein: Am. J. Cancer *28*: 63 (1937).
196. W. Cramer & E. S. Horning: Lancet *1*: 247 (1936); J. Path. & Bact. *44*: 633 (1937); Nature, London *139*: 196 (1937); Am. J. Cancer *37*: 343 (1939).
197. H. Burrows: J. Path. & Bact. *43*: 121 (1936).
198. A. Lacassagne & A. Raymond: Compt. rend. Soc. de biol. *124*: 183 (1937).
199. N. Dobrovolskaia-Zavadskaia: Compt. rend. Soc. de biol. *125*: 877 (1937).
200. N. Dobrovolskaia-Zavadskaia & P. Zéphiroff: Compt. rend. Soc. de biol. *128*: 971 (1939).
201. N. Dobrovolskaia-Zavadskaia & L. Pezzini: Compt. rend. Soc. de biol. *131*: 240 (1939).
202. L. Kreyberg & R. Eker: abstr. Am. J. Cancer *38*: 425 (1940).
203. J. S. Blaisdell, W. U. Gardner & L. C. Strong: Cancer Research *1*: 283 (1941).

Influence of adrenalectomy and of extracts of the whole gland on tumor growth. As has been stressed in the introductory remarks, the adrenal glands do not represent a functional entity, and hence the interpretation of experiments, in which the total gland has been removed, or its extracts have been administered, necessarily entails difficulties, since it must be doubtful whether the cortical or the medullar factors are responsible for the results obtained. This may also account for contradictory statements advanced on this subject. Some authors reported they had seen inhibition, or even cures of transplanted animal tumors, following adrenalectomy,[204, 205] while others failed to notice any influence of this interference on the tumor process.[188, 206] More recently, it has been stated that unilateral removal of an adrenal gland did not affect inoculated tumors, whereas bilateral adrenalectomy impeded the tumor growth,[191] but it is highly suggestive to associate this effect with the severe impairment of the general condition of the animals necessarily following this grave interference. The admixture of adrenal tissue to inoculated Jensen sarcoma has been claimed to depress the tumor growth,[207] and a growth inhibition of rat sarcomas, less so of rat carcinomas, was seen when the animals received injections of aqueous adrenal extracts.[187] Other investigators saw a tumor inhibiting effect from adrenal extracts, only when the glands employed were derived from animals previously treated with tumor material,[208, 209] an observation which, if substantiated, would be more closely related to immunological

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[204] H. Auler: Ztschr. f. Krebsforsch. *22*: 210 (1925).

[205] H. Flörcken: Ztschr. f. Krebsforsch. *24*: 465 (1927).

[206] F. Bischoff, L. C. Maxwell & H. J. Ullmann: Proc. Am. Soc. Biol. Chem. 25th meet., p. 80 (1931).

[207] J. Flaks: Ztschr. f. Krebsforsch. *30*: 145 (1929).

[208] F. Arloing, A. Josserand & J. Charachon: Compt. rend. Soc. de biol. *100*: 1035 (1929).

[209] F. Arloing, A. Morel, A. Josserand & A. Badinand: Compt. rend. Soc. de biol. *109*: 101 (1932); *112*: 156 (1933); Monit. Endocrinol. *2*: 207 (1934); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 72 (1935).

aspects than to endocrine aspects. But all these reports have been reduced in their significance in the light of the results of exact studies in which treatment with various preparations obtained from adrenal glands was found ineffective, as far as the growth of animal tumors is concerned.[210, 211]

Adrenocortical hormones. It has been claimed that injections of aqueous extracts of the adrenal cortex represent a very promising treatment in human neoplasia, and proof for this assumption should be furnished by the results obtained with this therapy in several thousand cancer cases, of which more than one-thousand had received a complete course of treatment. Of these, after five years 108 patients had survived and 52 of them were found at this time clinically free of cancer. Relief of pain and some improvements as to anemia and the nutritional state were reported to take place in the great majority of the treated patients.[212, 213] However, these remarkable results could not be confirmed in reexaminations and, moreover, animal experimentation failed to demonstrate similar beneficial effects on the tumor process from the administration of adrenal cortex extracts,[210, 211, 214, 215] or only a slight tumor inhibition was noted when large quantities of the glandular preparations were injected into the animals.[188, 216] For these reasons, the clinical treatment of neoplasms with adrenal cortex extracts must be considered with great reserve, since the subjective improvements observed were believed to represent merely a consequence of

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[210] W. H. Woglom: J. A. M. A. *95*: 473 (1930); Am. J. Cancer *15*: 704 (1931).

[211] K. Sugiura: Am. J. Cancer *15*: 707 (1931).

[212] W. B. Coffey & J. D. Humber: California & West. Med. *33*: 640 (1930); *44*: 160 (1936).

[213] J. D. Humber: 1st Internat. Cancer Congress, Madrid *2*: 44 (1933).

[214] K. Sugiura: Am. J. Cancer *15*: 128 (1931).

[215] S. Itami & E. McDonald: Science *72*: 460 (1930).

[216] G. Mazzone: Bol. soc. med.-chir. Catania *3*: 349 (1935); abstr. Ztschr. f. Krebsforsch. *43*: Ref. 33 (1936).

an increased tonicity,[217] due to the action of the hormone factors on carbohydrate and mineral metabolism and on the nervous system. It must be stressed that most of the experiments mentioned have been performed with adrenal extracts containing several endocrine factors and not with isolated hormones, which have been demonstrated to be present in the adrenal cortex and which have been shown to fulfill specific functions. In this respect, only one study is available in which desoxycorticosterone was injected into mice of an inbred strain with a known incidence of spontaneous mammary cancer, but this treatment failed to influence the time of the tumor appearance or the total tumor yield.[218]

Epinephrine. In discussing this hormone secreted by the adrenal medulla, which has acquired a pharmacological significance far exceeding its physiologic endocrine function, differentiation must be made between experiments in which this substance was injected locally into neoplasms, and those in which it was introduced into the body far from the site of the tumor. The first form of administration was generally found to result in tumor necrosis,[219-223] but there can be no doubt that this effect depended merely on the property of epinephrine of causing vasoconstriction, and hence of producing a local ischemia, impairing the blood supply of the tumors. This view seems supported by the failure to influence animal tumors by means of subcutaneous epinephrine injections,[223, 224] although a slight tumor inhibiting effect of this treatment has been reported by one research worker.[225] But, on the other hand, other authors observed even a stimulation of the growth of transplanted tumors,[188] or of the formation of tar cancer [226] following administration of epinephrine. Since insulin was found to exert an opposite influence (see below), it has been suggested that one may associate the action of epinephrine with its effect on the carbohydrate metabolism, i.e., the mobilization and cleavage of glycogen deposited in the liver and in the muscles, hence providing the neoplasms with needed nutritive material. But there is no evidence that this physiological mechanism is disturbed to any extent in neoplastic conditions, and rats with Jensen sarcoma were found to respond to epinephrine injections in the same way as tumor-free controls.[224] Another possibility of associating epinephrine with metabolic processes seemed to be given by the finding that quinone-like oxidation products of this substance represented potent respiratory activators,[227] but no effect on tumor growth resulted from injections of these epinephrine derivatives.[228]

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[217] R. H. Harris: J. A. M. A. *97*: 1457 (1931).

[218] M. B. Shimkin & H. G. Grady: J. Nat. Cancer Inst. *2*: 61 (1941).

[219] K. Reicher: Berlin. klin. Wchnschr. *47*: 987 (1910); Deutsche med. Wchnschr. *36*: 1356 (1910).

[220] Echtermeyer: Berlin. klin. Wchnschr. *48*: 1566 (1911).

[221] L. C. P. Ritchie: Lancet *2*: 1754 (1912).

[222] H. Engel: Ztschr. f. exper. Path. & Ther. *11*: 9 (1912).

[223] K. Sugiura & S. R. Benedict: J. Cancer Res. *14*: 487 (1930).

[224] G. Stolfi & E. Stolfi: Bol. soc. ital. di biol. sper. *11*: 918; 920 (1936).

6. *The Pancreas.*

Although the anatomical structure of this gland is complicated by the fact that it contains elements of both external and internal secretion, the problem of the pancreatic endocrine function may be considered as having been clarified to a highly satisfactory extent, since it has been discovered that the hormone production is localized in specific histologic configurations, viz., the Langerhans islets, and since the isolation of this endocrine factor, the insulin, was accomplished by the fundamental work of Banting and Best. Investigations

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225. F. Ludwig & J. v. Ries: Schweiz. med. Wchnschr. *64*: 141 (1934).
226. M. Kawamura & Y. Kamikawa: Tr. Jap. Path. Soc. *20*: 668 (1930).
227. B. Kisch & J. Leibowitz: Biochem. Ztschr. *220*: 97 (1930).
228. F. Arloing, A. Morel, A. Josserand & A. Badinand: Compt. rend. Soc. de biol. *114*: 631 (1933).

on other internal secretions supposed to be formed in the pancreas have not yet outgrown the initial experimental stage, and have not attained any essential significance in relation to the cancer problem.

Morphological findings. Pertinent studies are far from numerous, but they apparently agree in pointing to a hypertrophy of the pancreas islets in neoplasia, findings which have been obtained with human material,[229, 230] and with tumor-bearing mice.[231] In the latter communication, the view has been expressed that this phenomenon may be a consequence of the hyperglycemia, frequently accompanying the tumor process (cf. chapter II), and thus may represent a compensatory hypertrophy.

Insulin in neoplasms. Substances which are capable of lowering the blood sugar level have been found in tumor extracts,[232, 233] and the isolation of insulin from spindle cell sarcomas of rats has been reported.[234] The fact, however, that other authors failed to prove the presence of insulin-like factors in malignant tumors [235] indicates that this finding does not represent a constant quality of neoplasms. When, on the other hand, insulin has been isolated from liver metastases of a pancreatic carcinoma,[236] this isolation merely furnished proof for the functional activity of the said metastases, which histologically exhibited a structure resembling that of the Langerhans islets from which they obviously were derived.

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[229] M. Prates: Ztschr. f. Krebsforsch. *40*: 71 (1933).

[230] R. Reding: Compt. rend. Soc. de biol. *129*: 878 (1938).

[231] G. De Gaetani: Tumori n. s. *4*: 221 (1930).

[232] G. T. Cori: J. Cancer Res. *9*: 408 (1925).

[233] S. F. Gomes da Costa: Bol. inst. med. exper. para el estud. y trat. d. cáncer *12*: 619 (1935).

[234] A. H. Roffo & L. M. Correa: Ztschr. f. Krebsforsch. *24*: 488 (1927).

[235] W. Cramer, F. Dickens & E. C. Dodds: Brit. J. Exper. Path. *7*: 299 (1926).

[236] R. W. Cragg, M. H. Power & M. C. Linden: Arch. Int. Med. *60*: 88 (1937).

Similarly, the excessive production of insulin in some cases of pancreas carcinoma, clinically indicated by hypoglycemia, is a phenomenon characteristic only for this type of tumor, and is not associated with neoplasia in general. No sufficient evidence has been presented as yet for the claim that a conversion product of insulin, viz., the so-called aninsulin, should appear in the blood of cancer patients; aninsulin was reported to be obtainable in vitro, when insulin is incubated for several days in the presence of formol, and the resulting factor, it was found, had lost the hypoglycemic activity of insulin, but possessed antigenic property, as indicated by complement fixation tests with sera of rabbits which had received injections of aninsulin. Positive complement fixation for this factor was obtained with many sera of cancer patients, but not of tumor-free patients, but the specific nature of this phenomenon does not seem as yet convincingly proven.[237]

Influence of insulin on the tumor process. As remarked above, some authors were inclined to view the finding of hypertrophic Langerhans islets in the pancreas of animals or humans affected with neoplasia as being a defensive reaction of the organism, thus presupposing that insulin would exert an inhibitory action on the tumor growth. Additional support for such an opinion may be derived from the numerous experimental data described in detail in the chapters on organic chemistry, on nutrition, and on metabolism, which clearly indicate a tumor-promoting effect of carbohydrates ingested perorally or introduced parenterally, and consequently substances which cause hypoglycemia may be expected to influence tumor growth in the opposite manner. Actually, the results of animal experimentation seemed to confirm this theoretical assumption. An inhibitory effect on neoplastic growth of insulin administration preceding the tumor inoculation has been observed,[238] and a similar finding was obtained not only with the Ehrlich mouse carcinoma, but also regarding the tar cancer formation in mice.[239, 240] In the latter case, the transformation of precancerous lesions (warts) into malignant ones was found arrested, and an analogous action of insulin was seen in a study on the tar cancer development in rabbits.[241] As being in agreement with these results, a report may be considered according to which the destruction of the pancreas in fowl by means of cauterization stimulated the growth of transmitted Rous sarcoma.[242] Results of essentially the same nature have been obtained in numerous later studies which employed transplanted tumors,[242–252] or in those which dealt with tar cancer.[231, 233, 253] In one of these

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[237] Ph. Barral: Bull. Assoc. franç. p. l'étude du cancer *24*: 723 (1935); Compt. rend. Soc. de biol. *120*: 993 (1935).

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238. N. Waterman: Klin. Wchnschr. *4*: 1829 (1925).
239. F. Silberstein, J. Freud & T. Révész: Wien. klin. Wchnschr. *38*: 356 (1925).
240. F. Silberstein, J. Freud, T. Révész & B. Schneid: Ztschr. f. d. ges. exper. Med. *55*: 56 (1927).
241. H. D. v. Witzleben: Klin. Wchnschr. *4*: 2115 (1925).
242. S. Mori, M. Nakamura, S. Miyao & H. Nagase: Tr. Jap. Path. Soc. *20*: 663 (1930).
243. N. Piccaluga & S. Cioffari: Compt. rend. Soc. de biol. *93*: 1118 (1925).
244. J. Homma & T. Issiki: Tr. Jap. Path. Soc. *17*: 537 (1927).
245. Y. Ihara: Mitt. allg. Path. u. path. Anat. (Sendai) *7*: 103 (1931).
246. O. Lambret & J. Driessens: Compt. rend. Soc. de biol. *112*: 1430 (1933).
247. E. Fodor, G. Erös & St. Kúnos: Ztschr. f. Krebsforsch. *38*: 1 (1932).
248. St. Tokarski: Polski przegl. chir. *12*: 616 (1933); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 29 (1935).
249. O. Lambret, Ch. Grandclaude & J. Driessens: Compt. rend. Soc. de biol. *116*: 86 (1934).
250. O. Lambret & J. Driessens: Compt. rend. Soc. de biol. *119*: 589 (1935).
251. H. E. Eggers: Arch. Path. *18*: 507 (1934).
252. O. Lambret & J. Driessens: Bull. Assoc. franç. p. l'étude du cancer *25*: 77 (1936).
253. R. Münzner & F. Rupp: Deutsche med. Wchnschr. *51*: 1113 (1925).

investigations, performed with the Jensen rat sarcoma, insulin injections inhibited the tumor growth in about seventy per cent of the animals treated. Histologically, an increase of fibrous tissue was noticed in the retarded tumors; the liver exhibited a loss of glycogen; and a hypertrophy of the reticulo-endothelial elements appeared in the spleen of the animals, which effects were taken by the author as indications of an involvement of this system in the tumor inhibition.[252] But it is possible that this phenomenon was solely a secondary consequence—stimulation of the reticulo-endothelial system, due to the resorption of tumor decomposition products. On the other hand, mention must not be omitted of a number of reports, according to which insulin administration failed to affect the growth of animal tumors,[253-259] which divergent effects may find an explanation in the following fact. In one of the latter studies[253] it was stressed that simultaneously with the insulin administration the animals received a carbohydrate-rich diet, and it appears very probable that such a regime may lead to just the opposite of the desired effect, that is, to an accumulation of glycogen in the body, and hence to creation of conditions favorable for neoplastic growth.

These factors very likely also account for the, on the whole, unsatisfactory results obtained from insulin treatment in human neoplasia, because here the severe hypoglycemic state cannot, for obvious reasons, be maintained for a prolonged period of time, as would be necessary and can be tried in animal experiments. Accordingly, it has been stressed

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254. M. Händel & K. Tadenuma: Ztschr. f. Krebsforsch. *21*: 289 (1924).
255. F. Bischoff & L. C. Maxwell: J. Pharmacol. & Exper. Ther. *40*: 1 (1930).
256. R. Bauer & W. Nyiri: Wien. klin. Wchnschr. *38*: 353; 881 (1925).
257. A. Boivin: Compt. rend. Soc. de biol. *97*: 809 (1927).
258. Ph. Barral & R. Cade: Compt. rend. Soc. de biol. *101*: 1141 (1929).
259. H. Pinkerton, S. M. Beale, Jr., S. Warren & A. Kieling: Am. J. Cancer *27*: 99 (1936).

by authors [260, 261] who practiced the therapy in question with cancer patients, that the accomplishment of beneficial effects necessitates an administration of insulin doses and a restriction of carbohydrate ingestion which just avoids the reaching of the critical level of hypoglycemia. In this manner, sixteen inoperable cancer cases were treated and an improvement of the general condition of the patients and arrestation of tumor growth were almost regularly achieved. One of the neoplasms became operable, but only direct insulin injections into the tumor resulted in colliquation—an occurrence which, however, must be considered as undoubtedly entailing grave risks, on account of the sudden flooding of the body with toxic products liberated from the necrotic tumor masses. But, above all, the inadequateness of the insulin treatment of neoplasms has been best illustrated by the observation that, after cessation of the injections, the tumors frequently resumed a growth of fulminant character and, similarly, no permanent success has been obtained in other clinical studies in which cancer patients were treated with insulin.[262, 263] An exception is perhaps represented by the local insulin application in skin cancers, which treatment has been reported to result in cicatrization and sometimes cure of the tumors. An analogous effect was achieved with other substances causing hypoglycemia, such as guanidine derivatives (synthaline).[264] More experiences must, however, be collected to substantiate these claims, and the same applies as far as observations of an increased radiosensitivity of malignant tumors, following

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260. F. Silberstein: Schweiz. med. Wchnschr. *62*: 153 (1932).

261. F. Silberstein, J. Freud & T. Révész: Ztschr. f. d. ges. exper. Med. *55*: 78 (1927).

262. V. R. Stühlern: Wien. klin. Wchnschr. *39*: 685 (1926).

263. A. H. Roffo & J. Thomas: La chimie du cancer, Vigot frères, Paris 1933; p. 95.

264. S. F. Gomes da Costa: Compt. rend. Soc. de biol. *107*: 85 (1931); Bull. Assoc. franç p. l'étude du cancer *21*: 9 (1932); Tumori n.s. *6*: 140 (1932); Ztschr. f. Krebsforsch. *39*: 5 (1933); *41*: Ref. 252 (1935).

local[265] or parenteral insulin administration,[266-268] are concerned.

Insulin tolerance. In view of the therapeutical experiments with insulin in neoplasia, particular interest should be assigned to the analysis of the response of a tumor-bearing organism to this hormone but, regrettably, the investigations on this subject led to disturbing discrepancies, perhaps due to differences in the nutritional state of the animals or patients tested. An increased resistance against insulin in neoplastic conditions has been assumed to exist, on the basis of a number of experiments with animals or humans,[283, 289, 269-272] but other authors failed to notice any deviation from the normal response in this respect,[245, 253] or even observed an increased sensitivity toward insulin.[273] It must be mentioned that the findings of an increased insulin tolerance were obtainable in some of the aforementioned investigations[269, 270] only when the hormone was administered in protracted, small doses, but not when single higher doses were injected. A study, performed more recently, tested the blood sugar drop in nine cancer patients and seven cancer-free persons after insulin injection, and although the number of cases is comparatively small, the author interpreted the results as indicating a hypersensitivity of cancer patients against insulin, since in this disease the hypoglycemia appeared earlier, and reached a

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265. S. F. Gomes da Costa & F. B. Guedes: Compt. rend. Soc. de biol. *110*: 1051 (1932).

266. W. Goldschmidt: Wien. klin. Wchnschr. *46*: 1136 (1933).

267. F. Eichholtz & K. Baumgart: Arch. f. exper. Path. u. Pharmakol. *178*: 161 (1935).

268. W. Welchman: J. Med. Ass. South Africa *3*: 558 (1929); *7*: 395; 821 (1933); Libro de Oro, Buenos Aires 1935, p. 1495.

269. F. Silberstein, J. Freud & T. Révész: Biochem. Ztschr. *181*: 305 (1927).

270. S. Cioffari & N. Piccaluga: Tumori *12*: 387 (1927).

271. M. Perez: Bol. soc. ital. di biol. sper. *10*: 118 (1935).

272. J. La Barre & R. Loicq: Compt. rend. Soc. de biol. *129*: 876 (1938).

273. B. Borghi: Bol. soc. ital. di biol. sper. *2*: 6 (1927).

lower level (the decrease from the initial sugar value averaged thirty-five per cent), as compared with the reaction of tumor-free persons, in whom a drop of only fourteen per cent was found.[230] A final clarification of this problem would necessitate the testing of a much larger number of cases under exactly controlled experimental conditions. Otherwise, the effect of insulin administration to a tumor-bearing organism appears to exhibit no deviation from the normal, since it has been found to reduce the glycogen content of mouse tumors.[273] Such a diminution of the available carbohydrate may also account for the observation that insulin injections into sarcoma-bearing rats lowered the lactic acid content of their tumors,[160] whereas addition of the hormone in vitro did not affect the glycolysis of neoplastic tissue.[274]

Cancer and diabetes mellitus. The spontaneous phenomenon represented by the pathology of diabetes mellitus, which entails a typical insufficiency of the pancreatic internal secretion, gave opportunity for some studies on the interrelationship of this condition with simultaneously occurring neoplasia. Clinical observations, some of which go back to the beginning of this century,[275, 276] others of which are of more recent date,[277, 278] have shown that the development of cancer in patients, previously affected with diabetes, is followed by a disappearance, or diminution of the glycosuria. An interpretation of this phenomenon, however, is very difficult in the light of a report that, at the same time, the blood sugar level of these diabetic patients, who subsequently contracted malignancy, was not found to be changed. The decrease of the glycosuria cannot be due to renal factors, since suitable

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274. N. Waterman: Arch. neerl. physiol. *9*: 573 (1924).
275. J. Boas: Berlin. klin. Wchnschr. *40*: 243 (1903).
276. K. Oestreicher: Prag. med. Wchnschr. *28*: 293 (1903).
277. C. Wetzler-Ligeti & M. Köstenblatt: Wien. klin. Wchnschr. *49*: 1047 (1936).
278. W. Pfeifer: Monatschr. f. Krebsbekämpf. *6*: 241 (1938).

experiments with phlorizin showed a rather lowered renal threshold for glucose in these cancerous diabetics.[277] Furthermore, these findings appear to be hardly compatible with statements, according to which non-diabetic patients suffering from neoplasia exhibited a diminished glucose tolerance, i.e., glucose was excreted in the urine of these patients after sugar quantities were ingested which had no such effect in normal persons.[279, 280] Additional references to disturbances of the carbohydrate metabolism in neoplasia, as expressed in a hyperglycemia and in abnormal glucose tolerance tests, have been made in chapters II and VI, and it appears very likely that endocrine factors are not primarily involved in their etiology.

Other pancreatic factors. Brief mention may be made of other products of a supposed internal secretion of the pancreas, affecting the circulatory system, which have been tested in the form of various preparations (angioxyl, padutin, kallikrein), and which were reported to inhibit tumor growth.[281, 282] The quality of increasing cellular respiration has been ascribed to these factors. On the other hand, similar substances extracted from urine were seen to stimulate the growth of mouse carcinomas, when employed in the form of crude preparations, whereas purified extracts were found to be ineffective.[283] These divergencies doubtlessly reflect the insufficiently advanced knowledge of these alleged endocrine factors, the physiological significance of which is still obscure.

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279. R. Reding: Le terrain cancereux and cancérisable, Masson, Paris 1932.

280. D. Jackson & D. A. Todd: Texas State J. Med. *30*: 197 (1934).

281. St. Konsuloff & K. Dimitracoff: Ztschr. f. Krebsforsch. *36*: 37 (1932).

282. Ostendorf: Inaugural-diss., Münster 1932.

283. F. Bischoff & L. C. Maxwell: J. Pharmacol. & Exper. Ther. *52*: 378 (1934).

7. The Sex Glands.

Even aside from their endocrine functions, the gonads have for a long time attracted considerable interest from oncologists, who suspected these organs to be closely connected with the cancer problem. Such views have been stimulated, on the one hand, by the characteristic qualities of proliferation and of differentiation inherent in the generative cells of the sex glands whereas neoplastic tissues exhibit a powerful tendency to proliferation, but are extremely curtailed in their ability to differentiate. On the other hand, it was assumed that a possible causal relation between the predisposition to malignancy, which apparently increases with advancing age, and the senile involution of the generative glands may exist. Yet the construction of such a connection is actually based on a superficial interpretation of some clinical impressions, and is not adequately supported by scientific analysis. In this respect the fact should not be neglected, that incidence of neoplasms in persons of the younger age group is by no means uncommon and is not proved to be generally associated with an impairment of the sexual function. Furthermore, it is known that in very old age, that is, at a time of complete cessation of the sexual function, malignant tumors frequently take a much less rapid course than in younger individuals. These limiting remarks should not be understood as being intended to discredit, a priori, the significance of the sex glands for the tumor process, a problem which, as shown in the following, has led to comprehensive elaborations and to some very enlightening results. This relates especially to tumor formation in organs, the normal function of which is decisively determined by the sex hormones. On the basis of our present knowledge, grave doubt must be advanced as to the validity of opinions which would regard neoplasia as being primarily dependent on an overfunction or underfunction of the gonads. Nevertheless, views of this kind have been advanced, inasmuch as attention was drawn to an increased incidence of cancer claimed to exist in hermaphrodites, and the explanation of this predisposition to malignancy was seen in the deficient function of the sex glands in this condition.[284, 285] A similar trend of thought is exhibited in a theory, according to which the predominance of the manifest sexual character should be weakened with advancing age, and the resulting "bisexuality" of the organism should facilitate "amphimixis" of tissue cells, which in turn would mark the onset of malignant growth.[285] It is needless to say that this latter theory is of an entirely speculative nature and has not been substantiated by experimental evidence, which evidence could certainly not be easily furnished. But, in addition the statistical data pertaining to neoplasia in hermaphrodism, hardly can be considered as being sufficient proof for the conclusions derived from them.

Castration and tumor process. Numerous attempts have been made to contribute an experimental clarification to the problem dealt with in the preceding paragraph, by means of removing the sex glands in animals affected with tumors. These studies will be discussed prior to the detailed presentation of the interrelationship between neoplasia and the endocrinology of the gonads, separated according to sex. But it must be emphasized that in evaluating the results to be mentioned, it does not seem permissible to identify the effects of experimental castration with the senile involution of the sex glands. For the latter phenomenon represents a gradually progressing process associated with numerous other biological alterations, whereas castration is an abrupt interference, entailing the sudden interruption of the interrelationship of the sex glands with other organs of internal secretion, which

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284. H. Auler: Ztschr. f. Krebsforsch. *21*: 241 (1924).
285. L. Moszkovicz: Wien. klin. Wchnschr. *45*: 1529 (1932).

latter glands still possess at this time an unimpaired function. Furthermore, essential differences of effects of castration must be presupposed, in accordance with the experimental arrangements employed, because it is not irrelevant whether the interference is undertaken in animals prior to, or following attainment of sexual maturity, or before or after cessation of growth. Finally, no uniform behavior following castration seems to be exhibited by tumors of different genesis, viz., transplanted, spontaneous or chemically induced neoplasms. These factors may be responsible for the conflicting reports based on pertinent experiments, insofar as some authors interpreted their results as a stimulation of tumor growth,[35, 286-292] whereas others stated they had observed an inhibition in clinical experiences [293-297] or in animal experimentation.[298-301] In the latter respect, one worker stressed the fact that the resistance against subsequent tumor inoculation was increased in animals only when the castration was performed at an early age.[300] Another author found a marked difference in the growth of inoculated tumors, in accordance with the time interval which had elapsed between castration

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286. R. Graf: Zentralbl. f. allg. Path. u. path. Anat. *20*: 783 (1909).
287. J. E. Sweet, E. P. Corson-White & G. J. Saxon: J. Biol. Chem. *15*: 181 (1913).
288. J. S. Hilario: J. Exper. Med. *22*: 158 (1915).
289. T. Asada: Gann *16*: 35 (1922).
290. D. Engel: Ztschr. f. Krebsforsch. *19*: 339 (1923).
291. J. Maisin & L. Jacqmin: Compt. rend. Soc. de biol. *94*: 769 (1926).
292. A. Wagner: abstr. Ztschr. f. Krebsforsch. *38*: Ref. 55 (1932).
293. G. T. Beatson: Brit. M. J. *2*: 1145 (1901).
294. A. Thomson: Brit. M. J. *2*: 1538 (1902).
295. H. Lett: Lancet *1*: *227* (1905).
296. Guinard: abstr. Zentralbl. f. Chir. *32*: 487 (1905).
297. F. Cohen: Deutsche Ztschr. f. Chir. *99*: 415 (1909).
298. G. L. Rohdenburg, F. D. Bullock & P. J. Johnston: Arch. Int. Med. *7*: 491 (1911).
299. G. Joannovics: Beitr. z. path. Anat. u. allg. Path. *62*: 184 (1916).
300. J. B. Murphy & E. Sturm: J. Exper. Med. *42*: 155 (1925).
301. E. Pribram; Ztschr. f. Krebsforsch. *39*: 399 (1933); *42*: 368 (1935).

and tumor transplantation. A strong inhibition, or even regression, of the neoplastic growth, and simultaneously a severe damage of the tumor respiration, occurred when the tumors were transmitted comparatively soon after removal of the sex glands, but the opposite effect—stimulated tumor growth and increased oxygen consumption—was observed in animals, the castration of which had preceded the inoculation by a longer period of time.[301] As to the development of chemically induced tumors, the castration of animals does not seem to influence this process to a marked degree. In castrated female and male mice, following dibenzanthracene administration, tumors arose somewhat sooner than in control animals,[302] whereas castration of female rabbits slightly and irregularly inhibited the formation of tar cancer.[303] A more marked inhibition of tar cancer development was found in castrated female mice,[150] but the sarcoma formation following injection of methylcholanthrene into male mice was not influenced at all when the animals had been subjected to a prepuberty castration.[304] The author of the latter study comprehensively reviewed the conflicting results of previously published reports on this subject, and did not consider the evidence presented as convincing proof for the assumption of a direct influence of castration on the tumor process. Similar conclusions were drawn in another investigation, in which castration of mice of an inbred strain was found without marked effect on the takes and the growth of transplanted tumors, and it was believed that an inhibition of the neoplastic growth following castration, if observed in some experiments, had been due merely to an inhibition of the somatic growth caused by this interference.[305] Other authors, how-

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302. H. L. Stewart: Pub. Health Rep. *54*: 1026 (1939).
303. A. Tavares & E. Morais: Compt. rend. Soc. de biol. *125*: 179 (1937).
304. W. H. Woglom: Am. J. Cancer *40*: 321 (1940).
305. A, Rosenbohm; Ztschr, f. Krebsforsch, *51*; 164 (1941).

ever, expressed the opinion that castration would create conditions favorable for tumor development in the organism, support for which theory should be lent by the observation that malignant tumors developed in some instances in castrated animals following injections of embryonic tissue, or of cholesterol, or after painting with silver nitrate, all of which stimuli were not carcinogenic in control animals.[306] But since the tumors in question mostly arose far from the site of the agents administered, and since the small number of controls did not exclude the possibility of spontaneous tumor formation, the aforementioned experiments on carcinogenesis in castrated animals [302-304] must be regarded as being much more convincing. For the present, no decisive influence on tumor formation in general can be attributed to the extirpation of the sex glands. This statement, however, expressly excepts one special instance of neoplasia, namely of spontaneous mammary cancer in female mice belonging to certain inbred strains. As will be shown later, the ovarian secretion and other endocrine factors appear to be closely connected with this type of tumor, and accordingly spaying of the animals has been found decisively to influence the appearance of these tumors. These relations, as well as other phenomena occurring in secondary male or female sex organs, will be discussed in the following presentation dealing separately with the male and female gonads.

A. Testis.

Morphological findings and hormone excretion. On the basis of histological examinations in twenty-one cancer patients, it has been claimed that a hyperplasia of the interstitial testicle cells took place in eighty per cent of the cases tested.[307] But subsequent studies failed to confirm this finding, inasmuch as no typical change of the testicular histology was seen excepting a frequently occurring atrophy of the seminal tubules associated with unimpaired spermiogenesis,[308] or instead unspecific alterations were observed in the testicles of cancer patients, similar to those encountered in other grave diseases.[309] In accordance with this failure to prove anatomically deviations of the testicular function in neoplasia, no support for such an assumption has been furnished by the few analyses of the excretion of testis hormones in this disease. No appreciable difference from the normal has been found as to the quantity of urinary androgens in four investigated cases of breast cancer.[310] A report has been made on the presence of comparatively large amounts of an androstadiene in the urine of a male patient suffering from a malignant tumor of the adrenal,[311] but this finding is obviously not connected with malignancy in general, but rather with the type of tumor in question. A similar interpretation may be applied to data pertaining to patients suffering from tumors of the testis proper. A marked reduction of the urinary excretion of androgens was found in patients with seminomas, and patients with chorionepitheliomas of the testis also showed a diminution of these hormones in the urine, though to a lesser degree. The simultaneous increase of hypophysial gonadotropins, which was detected in the urines of patients with testis tumors, indicated the existence of a hypofunction of the testis in these cases.[311a]

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[306] F. Sauerbruch & E. Knake: Ztschr. f. Krebsforsch. *44*: 223 (1936).
[307] E. Sehrt: Zentralbl. f. allg. Path. u. path. Anat. *54*: 353 (1932).

Influence of testicular extracts and testis hormones. Several authors have reported that the administration of testicular extracts or tissue, frequently coinciding with the tumor inoculation, inhibited the growth of animal tumors,[301, 312, 313] but in other investigations such an effect was not observed,[314] or even a growth stimulation of tumors was seen.[315] It must be borne in mind that these results do not necessarily represent true endocrine effects, i.e., they cannot be associated exclusively with the action of male sex hormones, since in most of these studies preparations were employed which contained, in addition to the endocrine factors, a number of undefined substances which might affect growth processes in an unspecific manner. The same interpretation must be applied to some clinical attempts at testicular therapy in neoplastic conditions, which attempts were made at a time prior to the availability of isolated or synthetic testis hormones. Such experiments of administering testicular extracts to cancer patients were undertaken by one author for the reason that Abderhalden's tests seemed to indicate a damaged testicular function in cancer patients, but no beneficial effects of this treatment were reported.[316] On the other hand, some investigators in their work proceeded from the assumption of a tumor promoting action of the testis, and attempted to treat cancer patients with immune sera derived from animals injected with testicular tissue. Favorable results of this treatment have been reported and were regarded as being due to an antibody present in the immune sera which was directed against the nuclear chromatine.[317, 318] Hence these experiments have no direct connection with endocrine factors, but

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[308] W. Beck: Zentralbl. f. allg. Path. u. path. Anat. *65*: 65 (1936).

[309] E. E. Collins: Arch. Path. *22*: 470 (1936).

[310] M. Ross & R. I. Dorfman: Cancer Research *1*: 52 (1941).

[311] H. Burrows, J. W. Cook, E. M. F. Roe & F. L. Warren: Biochem. J. *31*: 950 (1937).

[311a] Ch. Hamburger & E. Godtfredsen: Acta path. et microbiol. Scandinav. *18*: 484 (1941).

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312. H. Elsner: Ztschr. f. Krebsforsch. *23*: 28 (1926).

313. U. Bertolotto: Sperimentale *85*: 331 (1931); abstr. Ztschr. f. Krebsforsch. *35*: Ref. 155 (1932).

314. F. Prime & C. D. Haagensen: Am. J. Cancer *20*: 630 (1934).

315. D. C. Hoffmann, F. Parker, Jr. & Th. T. Walker: Am. J. Path. *7*: 523 (1931).

316. L. Maiditsch: Ztschr. f. d. ges. exper. Med. *76*: 738 (1931).

317. Deutschmann: Klin. Wchnschr. *1*: 398 (1922).

318. Kotzenberg: Beitr. z. klin. Chir. *126*: 226 (1922).

must be considered as being of immunological nature; furthermore, the effectiveness of this treatment has not been sufficiently substantiated to warrant continued application. Likewise only doubtful results have been obtained by means of another indirect interference with the testicular function, that is, the vasectomy (ligation of the spermatic duct). This procedure seemed to promote the growth of animal tumors [301] whereas, clinically, temporary subjective improvements were reported.[319]

Much more light has been shed on the problem under discussion by work of more recent date in which pure preparations of testis hormone were used. In this connection, one fact has been established, which fact may attain significance in conjunction with the action of estrogens on tumor formation. For it has been found that injections of testosterone into female mice of strains exhibiting a high mammary cancer incidence to a marked degree impeded the appearance of the tumors, when the treatment was started at an early age of the animals.[320-322] Tumors which had already developed were not influenced by the male sex hormone,[320-322] and therefore it may be assumed that this endocrine factor counteracts the tumor formation by shifting the hormone equilibrium in a direction which is unfavorable for neoplasia, especially by means of cancelling the opposite influence of estrogens. Such an assumption of an interrelationship with other endocrine mechanisms is also supported by the finding that injections of testosterone propionate lowered the incidence of spontaneous mammary cancer only in virgin female mice, whereas this effect was not obtained in animals with a normal breed-

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319. H. Steindl: Arch. f. klin. Chir. *130*: 117 (1924).

320. I. T. Nathanson & H. B. Andervont: Proc. Soc. Exper. Biol. & Med. *40*: 421 (1939).

321. A. Lacassagne & A. Raynaud: Compt. rend. Soc. de biol. *131*: 586 (1939).

322. E. B. Jones: Cancer Research *1*: 787 (1941).

ing history.[322] As far as transplanted tumors are concerned, injections of androgens extracted from urine have been reported markedly to inhibit the metastasis formation of Brown-Pearce carcinomas of rabbits, and also to prolong the life of the animals, whereas testosterone injections were less effective in this respect.[323] Chemically induced tumors appear to be affected to a much lesser degree by testis hormones. Injections of testosterone propionate slightly delayed the tumor development in mice painted with methylcholanthrene;[324] in further experiments, it was ascertained that this effect was obtainable only when high hormone doses and small amounts of the carcinogen were employed, and that the inhibitory action of the hormone mainly concerned the transformation of papillomas into malignant tumors.[325] Other authors failed to notice any significant influence of testosterone administration on the tumor formation by means of benzpyrene in mice,[326] and it must be concluded from this study, and from the aforementioned experiments, that the tumor-inhibiting action of the male sex hormone is only very limited, excepting in those instances, discussed above, where it may counteract the effect of other endocrine factors. Under special conditions, it even may promote the neoplastic process, as was observed in a study on a transplantable mammary fibroadenoma of rats. In this case, injections of androgens, though decreasing the number of takes in treated animals, enhanced the transformation of this benign tumor into a sarcoma.[327] In human pathology, the male sex hormone appears to exert a very

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323. J. R. Murlin, C. D. Kochakian, C. L. Spurr & R. A. Harvey: Science *90*: 275 (1939); Arch. Path. *28*: 777 (1939).

324. J. Flaks & A. Ber: Compt. rend. Soc. de biol. *128*: 506 (1938).

325. J. Flaks & A. Ber: Bull. Assoc. franç. p. l'étude du cancer *28*: 108 (1939).

326. J. Maisin, Y. Pourbaix & G. Rijckaert: Compt. rend. Soc. de biol. *130*: 209 (1939).

327. J. Heiman: Am. J. Cancer *39*: 178 (1940).

marked influence on one particular type of neoplasm, viz., the prostatic cancer. Patients suffering from this disease showed a marked increase of the serum acid phosphatase following injections of androgens, a phenomenon indicating a stimulation of the neoplastic process (cf. chapter IV). On the contrary, administration of estrogens to, or castration of patients with, prostatic cancer depressed the activity of the serum acid phosphatase—i.e., these measures apparently counteracted the tumor growth.[328] A report on twenty-one patients with prostatic carcinoma, in whom bilateral castration was performed, recorded an appreciable improvement in fifteen cases, and a considerable decrease of the serum acid phosphatase in thirteen cases; gain of weight, increase of appetite, increase of the red blood count, shrinkage of the primary lesions and an increased roentgenological density of bone metastases were observed in several of these patients.[328a] It was concluded from these observations that the elimination of androgens, although not affecting a cure, often greatly inhibits the course of prostatic cancer.[328b] Other authors also found beneficial effects in prostatic cancer, resulting from bilateral orchiectomy [328c] or from irradiation of the testes.[328d] These phenomena may be easily understood in the light of the fact that prostatic tumors originate from an organ, the growth of which is physiologically stimulated by the male sex hormones, and also that neoplasms may respond to endocrine stimuli in a manner similar to that of the normal parent tissue from which they arose. Therefore, it may again be emphasized that endocrine factors should not

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328. C. Huggins & C. V. Hodges: Cancer Research *1*: 293 (1941).

328a. C. Huggins, R. E. Stevens, Jr. & C. V. Hodges: Arch. Surg. *43*: 209 (1941).

328b. C. Huggins, W. W. Scott & C. V. Hodges: J. Urol. *46*: 991 (1941).

328c. L. F. Ajamil: Bol. Liga contra el cancer *16*: 329 (1941); abstr. J. A. M. A. *118*: 1166 (1942).

328d. A. D. Munger: J. Urol. *46*: 1007 (1941).

be expected to affect neoplastic processes of various types and etiologies in a uniform manner. The resulting effects must largely depend on the physiological interrelationship of the hormones with other organs in general, and with interferences with the endocrine equilibrium in particular.

B. Ovary.

The interaction between the female sex gland and its internal secretion and the neoplastic process has formed the subject of an exceedingly large number of investigations, resulting in a comprehensive elaboration of this problem and leading to some very significant results. On account of the scope of the literature on the subject, the following survey does not claim to be a complete enumeration of all studies undertaken in this field, especially as far as references of older dates are concerned. Supplementary information may be derived from a comprehensive review presented in 1939 by Gardner,[329] as well as from other treatises and summaries on estrogens and neoplasia, recently published.[330–333a] These publications must be regarded as being all the more valuable, since most of the authors had actively participated in experimental work on the problem in discussion, and had made important contributions.

Morphological findings, and functional activity of the ovary in neoplasia. It has been reported that inoculation of tumors to female mice was followed by structural and functional damages of the ovaries, but an unspecific cause, i.e., the toxic action of products of tumor breakdown, was held

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329. W. U. Gardner: Arch. Path. *27*: 138 (1939).
330. A. Lacassagne: Am. J. Cancer *37*: 414 (1939).
331. H. Friedrich-Freksa: Ber. ges. Gynäk. u. Geburth. *40*: 225 (1940).
332. L. Loeb: J. Nat. Cancer Inst. *1*: 169 (1940).
333. R. R. Greene & J. I. Brewer: Am. J. Roentgenol. *45*: 426 (1941).
333a. E. Allen: Endocrinology *30*: 942 (1942).

to be responsible for these phenomena.[334-338] Comparisons of the estrous cycles of mice, belonging to strains with great or slight susceptibility to spontaneous breast cancer respectively, did not reveal any significant difference in the behavior of these animals, and also did not furnish any evidence for an increased estrogen secretion in the high-mammary-tumor-strains.[339, 340] When tumor-bearing female mice were subjected to castration, it was observed that a period of completely absent estrus was followed by a period of continuous estrus and since a similar phenomenon occurred after implantation of normal tissue, it was believed that this was due to products of tissue breakdown which stimulated the internal secretion of pituitary gonadotropic factors.[341] A similar reason may explain the histological finding of an overfunction in most of the ovaries which had been removed surgically from thirty-five patients suffering from uterine cancer.[342] No decision can yet be made as to what extent these factors may be responsible for the observation of the markedly increased content of estrogenic substances in the blood of cancer patients, even of male ones, reported by several authors.[343, 344] This finding has more recently been confirmed for the blood of patients afflicted with cancer of the uterine cervix, and an augmented urinary excretion of gonadotropic substances was

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334. G. L. Rohdenburg & F. D. Bullock: J. Med. Res. *33*: 147 (1915).
335. E. Pribram: Ztschr. f. Krebsforsch. *35*: 336 (1932).
336. E. Allen, A. W. Diddle, L. C. Strong, T. H. Burford & W. U. Gardner: Am. J. Cancer *25*: 291 (1935).
337. B. Kutscherenko & A. Issachanow: Acta Cancrol. *1*: 379 (1935).
338. H. Druckrey: Arch. f. exper. Path. u. Pharmakol. *180*: 367 (1936); Ztschr. f. Krebsforsch. *48*: 241 (1938).
339. E. L. Burns, M. Moskop, V. Suntzeff & L. Loeb: Am. J. Cancer *26*: 56 (1936).
340. A. Brunschwig & A. D. Bissel: Arch. Surg. *33*: 515 (1936).
341. E. Pribram & M. Raschke: Ztschr. f. Krebsforsch. *44*: 93 (1936).
342. C. Daniel & A. Babès: Libro de Oro, Buenos Aires 1935, p. 264.
343. E. Dingemanse, J. Freud, S. E. de Jongh & E. Laqueur: Arch. f. Gynäk. *141*: 225 (1930).
344. P. Engel: Ztschr. f. Krebsforsch. *34*: 658 (1931).

also found in these cases.[345] Furthermore, it has been claimed that the urinary excretion of estrogenic factors was increased in female cancer patients, a phenomenon which disappeared after radical operation of the tumor, but reappeared in the case of recurrences.[346] Other authors, however, were not able to demonstrate deviations from the normal, as to excretion of estrogens in four cases of breast cancer,[310] and the presence of large quantities of estrogenic hormones in the urine of a male patient suffering from a malignant adrenal tumor [311] most probably was caused by this particular type of neoplasm. In a study of recent date, it has been stated that a purple pigment can be extracted from urines of male and female cancer patients, a pigment which was only rarely present in the urine of cancer-free persons, and it was considered to represent a conversion product of estrogens, with markedly decreased potency. In the course of this investigation, it was further observed that administration of estrogens, or of progesterone, to patients with cancers of the female genital tract, resulted in a lesser estrogen excretion, as compared with the reaction of cancer-free women, and it was supposed that the estrogenic substances ingested were transformed by the cancer patients into less active derivatives.[347] In continuation of this work, in pooled urines of noncancerous, or of cancerous persons respectively, the estrogenic substances were separated into three fractions, representing presumably estrone, estradiol and estriol. The total estrogenic activity was found to be higher in the urines of noncancerous women than in cancerous ones, whereas the opposite findings were obtained from a comparison of the urines of noncancerous and cancerous men. The "estriol" fraction showed a higher titer in the urines of noncancerous women than in those of cancerous ones. On the other hand, the titer of the "estradiol" fraction isolated from the urines of cancerous men surpassed the activity of the analogous fraction derived from noncancerous men. On the basis of these data, it was held likely that assays of the fractions named may yield more valuable information than determinations of the total estrogenic activity.[347a] In this connection, reference is also made to a study, according to which spayed female mice of a high-tumor-strain appeared to be less susceptible to the action of estrogens than animals of a low-tumor-strain, but the reason for this behavior was assumed perhaps to lie in the fact that mice of a strain with high susceptibility to spontaneous breast cancer normally produced larger estrogen amounts.[348] Rats bearing transplanted tumors were found to respond to estrogen administration in the same way as cancer-free animals.[338]

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[345] R. Imbert, M. Mosinger & H. Bontoux: Compt. rend. Soc. de biol. *122*: 692 (1936).

[346] I. Schön: Thesis, Kiel 1934; abstr. Ztschr. f. Krebsforsch. *43*: Ref. 34 (1936).

[347] G. Pincus & M. Graubard: Endocrinology *26*: 427 (1940).

Estrogenic substances in tumors. Several reports are available, according to which an increased content of female sex hormones has been found to be present in various types of tumors of animals or humans.[349–353] But in evaluating these results it must not be overlooked that the customary methods for the determination of estrogenic substances consist of biological tests, which are by no means specific for the products of the ovarian internal secretion since a number of organic substances, which occur naturally, or are prepared synthetically, have been shown to be capable of eliciting the same biological effects as the true hormones of the ovary. A similar

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347a. G. Pincus & W. H. Pearlman: Cancer Research *1*: 970 (1941).

348. P. J. van Gulik & R. Korteweg: Am. J. Cancer *38*: 506 (1940).

349. C. F. Cori: J. Exper. Med. *45*: 983 (1927).

350. S. Loewe, W. Raudenbusch & H. E. Voss: Biochem. Ztschr. *249*: 443 (1932).

351. J. Kosakaé, T. Ohga & S. Okamoto: Jap. J. Obst. & Gynec. *16*: 299 (1933).

352. D. Lewis & C. F. Geschickter: J. A. M. A. *103*: 1212 (1934).

353. J. Heiman & O. F. Krehbiel: Am. J. Cancer *27*: 450 (1936).

interpretation may be applied to the aforementioned observations of an increased estrogen content in the blood or the urine of cancer patients. Furthermore, it has been stressed that findings of estrogenic factors in malignant tumors can be adequately evaluated only if these values are compared with those obtained in normal tissues, since considerable quantities of this hormone have been demonstrated as being present, e.g., in the psoas muscles of normal mice.[354] Such a requirement appears to have been fulfilled in a study in which the estrogen content of normal breasts was compared with that of the mammary glands of patients suffering from breast cancer, and markedly higher values were obtained for the latter ones.[355] But on the other hand, an investigation of more recent date led to the conclusion that the concentration of estrogens in human neoplasms varied widely. Similar findings were obtainable in various normal tissues,[356] and in spontaneous mammary cancer of rats no estrogenic substances were detectable.[357] Hence, one does not seem justified in generally assuming the presence of increased concentrations of estrogenic factors in malignant tumors.

Ovariectomy and tumor process: While in the preceding section, dealing with the effects of castration, it has not been possible to interpret the available evidence to prove a tumor inhibition as resulting from this interference, supplementary data which have clearly established such a phenomenon in one particular instance of tumor formation must be mentioned here. The susceptibility to spontaneous mammary cancers in female mice of certain inbred strains could be lowered to a marked degree when the animals underwent spaying at an early age.[329, 352, 358-360] Compared with a cancer incidence of eighty per cent in normal breeding animals, a frequency of only seventeen per cent has been observed in ovariectomized females, and hence it may be supposed that products of ovarian secretion are essentially involved in this form of neoplasia. However, in the light of the results mentioned previously, only a lesser significance can be attached to reports which claim that bilateral ovariectomy of mice or rats also increased the resistance of animals against subsequent inoculation of tumors,[361-363] and the variable results obtained with chemically induced tumors in spayed animals have been quoted before. Nevertheless, several clinicians believed that removal of the ovaries may represent a valuable palliative measure in cases of inoperable breast cancer, and they reported on beneficial effects from such procedures.[293-297, 364-367] Likewise, roentgen sterilization, as a substitute for the surgical ovariectomy has been advocated by many authors as being a promising means of preventing recurrences and metastases following radical operations of breast cancers, especially in younger persons.[cf. 368]

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[354] R. T. Frank, M. A. Goldberger, U. J. Salmon & R. Friedman: Proc. Soc. Exper. Biol. & Med. *32*: 1665 (1935).

[355] A. Lacassagne & W. Nyka: Compt. rend. Soc. de biol: *116*: 844 (1934).

[356] M. Nakatani: Gann *30*: 434 (1936).

[357] A. W. Wright, G. H. Klinck & J. M. Wolfe: Am. J. Path. *16*: 817 (1940).

The influence of estrogenic substances on neoplasia. The experimental data, discussed in the preceding sections, war-

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[358] L. Loeb: Internat. Conf. Cancer, London 1928, p. 48.

[359] W. S. Murray: J. Cancer Res. *12*: 18 (1928).

[360] L. Loeb, E. L. Burns, V. Suntzeff & M. Moskop: Canad. M. A J. *35*: 117 (1936); Proc. Soc. Exper. Biol. & Med. *35*: 320 (1936).

[361] E. Pribram: Ztschr. f. Krebsforsch. *34*: 544 (1931).

[362] Y. Yano: Acta dermat. *22*: 140 (1933).

[363] Y. Nitta: Jap. J. Obst. & Gynec. *19*: 90 (1936).

[364] J. T. Witherspoon: Am. J. Obst. & Gynec. *31*: 173 (1936).

[365] G. G. Smith: Am. J. Roentgenol. *36*: 65 (1936).

[366] T. Pissareva & I. Deinéka: Eksper. Med. *1*: 49 (1936); abstr. Ztschr. f. Krebsforsch. *44*: Ref. 145 (1936).

[367] J. A. Schockaert: Bull. soc. belge Gynec. *11*: 130 (1935).

[368] I. I. Kaplan: Radiation therapy, Oxford University Press, New York 1937; pp. 399, 400.

rant, in spite of some conflicting statements, interest in the significance of the ovarian internal secretion in connection with the neoplastic process, and a very large number of investigations have consequently been devoted in particular to the hormones produced in the graafian follicles, which are known under the general designation of estrogens and are available in the form of numerous commercial preparations. Similar relations are apparent in this case as in those connected with the effect of ovariectomy, inasmuch as satisfactory agreement has been reached on a definite effect obtained from the administration of estrogenic factors to female animals which belong to inbred strains exhibiting a high susceptibility to spontaneous mammary cancer. The frequency of the formation of tumors was found to be markedly increased, the time of their appearance was accelerated [369-371] and, in animals of strains which were refractory to spontaneous cancer development, mammary tumors also arose more frequently following prolonged treatment with estrone.[372] Several reports have been presented, which also indicate a growth-promoting effect from the administration of estrogens on various transplanted animal tumors,[367, 373-375] and the observation of an increased tumor yield of Rous sarcoma transmitted to fowl during the egg-laying period has been associated with a stimulated ovarian function during this time.[376] Variations, however, in the transplantability of

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369. V. Suntzeff, E. L. Burns, M. Moskop & L. Loeb: Am. J. Cancer *27*: 229 (1936).

370. A. Lacassagne: Compt. rend. Acad. d. sc. *195*: 630 (1932); Bull. Assoc. franç. p. l'étude du cancer *27*: 96 (1938).

371. C. S. McEuen: Am. J. Cancer *34*: 184 (1938).

372. G. M. Bonser, L. H. Stickland & K. I. Connal: J. Path. & Bact. *45*: 179 (1937).

373. T. B. Robertson & T. C. Burnett: J. Exper. Med. *17*: 344 (1913); *23*: 63 (1916).

374. A. Fornero: Tumori n.s. *1*: 255 (1927).

375. L. Gross: Ztschr. f. Krebsforsch. *36*: 606 (1932).

376. E. Fränkel: Ztschr. f. Krebsforsch. *32*: 167 (1930).

chemically induced fowl sarcomas, though depending on the season, could not be definitely associated with corresponding changes in the function of the ovary.[377] Estrogen treatment was found to increase in male rabbits the susceptibility to Brown-Pearce carcinoma, and to enhance its growth and metastasis formation.[378] A stimulating effect of estrogenic substances has been ascertained regarding the transplantability and the growth of a benign mammary fibroadenoma of rats, but it was stressed that this effect failed to occur, once the fibroadenoma had become malignant. From this observation the conclusion was drawn that the benign tumors responded to the hormone in the same way as the normal mammary gland, whereas the subsequently developing sarcoma was less affected by this physiological stimulus.[379] This agrees well with the general concept, described in detail later, which envisages the stimulation of neoplastic processes by estrogens as being principally confined to the female sex organs and to the mammary gland, i.e., to organs the functional activity of which is normally regulated by the endocrines of the ovary. Such a relationship may explain findings, according to which estrogens failed to influence the growth of transplanted animal tumors,[128, 380–382] or even exerted an inhibitory effect.[363, 383, 384] In the latter study,[384] performed with a transplantable mammary adenocarcinoma of rats, it has been observed that the inhibition of tumor growth was accompanied by lactational phenomena in the breast, and it was supposed that this transformation into the functioning gland may have been responsible for suppressing proliferation. As mentioned above, administration of estrogen seemed to decrease the malignancy of human prostatic cancer, inasmuch as a diminished activity of the serum phosphatase may, in this condition, be taken as a measure of the tumor virulence.[328-328d] These results obviously demonstrate that estrogens do not affect various neoplastic processes in a uniform manner, and this conclusion was also drawn as the result of an investigation, according to which estrogens failed to influence inoculated mouse tumors, but increased the susceptibility of the skin of animals to the carcinogenic stimulus of benzpyrene, resulting in an earlier appearance of tumors in them than in control animals.[385]

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[377] P. R. Peacock: Am. J. Cancer *25*: 37 (1936).

[378] J. W. Mu: Am. J. Cancer *34*: 407 (1938).

[379] F. E. Mohs: Proc. Soc. Exper. Biol. & Med. *43*: 270 (1940).

[380] K. Molnar: Ztschr. f. Krebsforsch. *38*: 188 (1932).

[381] L. A. Emge, K. M. Murphy & W. Schilling: Proc. Soc. Exper. Biol. & Med. *38*: 21 (1938).

[382] K. M. Murphy, W. Schilling & L. A. Emge: Proc. Soc. Exper. Biol. & Med. *39*: 298 (1938).

[383] W. T. Salter, I. T. Nathanson & H. Wilson: Cancer Research *1*: 60 (1941).

[384] M. J. Eisen: Cancer Research *1*: 457 (1941).

The numerous studies, which will now be considered, advance one step further by attempting to prove not only a tumor-promoting activity, but also a true carcinogenic activity of estrogenic hormones. They have been prompted not only by several of the aforementioned findings, but also by the fact, referred to in the second chapter, that the chemical configuration of the sex hormones (male and female) exhibit a certain resemblance to that of some carcinogenic hydrocarbons, inasmuch as both groups of substances contain the phenanthrene nucleus. Prolonged administration of estrogens to mice was seen to produce a strong keratinization of the vagina and metaplastic changes in the uterine mucosa of females, or in the prostatic gland of males. Whereas in these earlier experiments no formation of true malignant tumors was observed,[386-388] in later studies the proliferation of the vagina and cervix epithelium was found to result in cancer development in approximately ten per cent of the treated animals.[389] Sarcoma formation has been observed in estrogen injected mice,[390] mammary carcinoma developed after this treatment in rats of a strain which was completely free of spontaneous mammary tumor incidence,[391] and cervical cancers, which could be successfully transplanted, were produced in mice by means of prolonged estrogen administration.[392] Pathologies suspected of being cancer have been induced in the uterus of rabbits treated simultaneously with tar and with estrogen injections.[393] Definitely malignant uterine tumors were seen in castrated rabbits treated in a similar manner,[394] and in a later study uterine tumors were induced in castrated rabbits without employing tarring.[395] A combination of two stimuli has also been used by another author who observed the formation of uterine and mammary cancers in mice, the skin of which was painted with dibenzanthracene and into which estrone was injected at the same time.[396] Another series of experiments succeeded in eliciting malignant tumors by means of estrogens when large amounts of the substances were administered to male mice of strains, the females of which showed a high susceptibility to spontaneous mammary tumors,[370, 397-399] and analogous results from the hormone ad-

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[385] M. D. Gilmour: J. Path. & Bact. *45*: 179 (1937).

[386] A. Lacassagne: Compt. rend. Soc. de biol. *113*: 590 (1933).

[387] H. Burrows & N. M. Kennaway: Am. J. Cancer *20*: 48 (1934).

[388] H. Selye, D. L. Thomson & J. B. Collip: Nature, London *135*: 65 (1935).

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389. V. Süntzeff, E. L. Burns, M. Moskop & L. Loeb: Am. J. Cancer *32*: 256 (1938).

390. A. Lacassagne: Compt. rend. Soc. de biol. *126*: 190; 193 (1937).

391. C. F. Geschickter: Science *89*: 35 (1939).

392. W. U. Gardner, G. M. Smith & L. C. Strong: J. A. M. A. *110*: 1182 (1938).

393. M. Borst: Wissenschaftl. Woche, Frankfurt a. M., *2*: 1 (1934).

394. H. Pierson: Ztschr. f. Krebsforsch. *41*: 103 (1935).

395. H. Pierson: Ztschr. f. Krebsforsch. *46*: 109; *47*: 1; 336 (1937).

396. I. H. Perry: Proc. Soc. Exper. Biol. & Med. *35*: 325 (1936).

397. W. U. Gardner, G. M. Smith, E. Allen & L. C. Strong: J. A. M. A. *107*: 656 (1936); Arch. Path. *21*: 265 (1936).

398. W. U. Gardner, G. M. Smith, L. C. Strong & E. Allen: Arch. Path. *21*: 504 (1936).

399. W. Cramer & E. S. Horning: Lancet *1*: 247 (1936).

ministration were observed in castrated male mice.[400, 401] In the course of these experiments, histological changes in the prostatic glands have been noted, which has given rise to the supposition that increased estrogen production may be involved in the formation of human prostatic cancer.[400] Development of hypophysial tumors in mice and rats has also been observed following estrogen treatment.[399, 401, 402] Instead of prolonged injections of estrogens, single implantations of estrone pellets have been successfully used for the production of mammary cancer in male mice of a high tumor strain,[403] and a similar procedure elicited mammary tumors in rats, although these tumors did not exhibit a tendency to invasive growth in spite of intensive proliferation [404] and sometimes disappeared after removal of the pellets.[405] These findings suggest a quasi-benign nature of the growth induced by estrogens and, indeed, a number of other experiments yielded analogous results. Only an atypical epithelial growth of the cervix was produced in monkeys subjected to estrone injections and to local mechanical traumas.[406] Hyperplastic and metaplastic changes of the uterine mucosa were obtained in estrogen treated guinea pigs; [407] and connective tissue tumors of probably benign character were produced in the uterus of guinea pigs by means of injections or implantation of estradiol benzoate.[408] These findings, as well as clinical analyses, make it appear probable that an overproduction of estrogenic hormone is responsible for the development of benign tumors of the uterus or of the breast in human pathology.[409] The relative effectiveness of various estrogenic substances has been compared [329, 330, 410] and, on the basis of extensive experimentation, valuable analyses of the factors involved in the tumor induction by means of estrogens have been presented.[410a, 410b]

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[400] H. Burrows: Am. J. Cancer *23*: 490; *24*: 613 (1935).

[401] C. S. McEuen, H. Selye & J. B. Collip: Lancet *1*: 775 (1936).

[402] B. Zondek: Lancet *1*: 776 (1936); Am. J. Cancer *33*: 555 (1938).

[403] G. H. Twombly: Proc. Soc. Exper. Biol. & Med. *40*: 430 (1939).

[404] R. L. Noble, C. S. McEuen & J. B. Cóllip: Canad. M. A. J. *42*: 413 (1940).

[405] R. L. Noble & J. B. Collip: Canad. M. A. J. *44*: 1 (1941).

[406] M. D. Overholser & E. Allen: Surg., Gynec. & Obst. *60*: 129 (1935).

[407] W. O. Nelson: Endocrinology *24*: 50 (1939).

[408] A. Lipschütz & L. Vargas, Jr.: Compt. rend. Soc. de biol. *129*: 519; 524 (1938); Lancet *1*: 1313 (1939).

It is of great interest that a synthetic estrogen of high potency, viz., stilbestrol, has been found capable of inducing mammary adenocarcinomas after subcutaneous or peroral administration, or of causing testicular tumors, when applied locally in the form of pellets.[411] Similarly, several derivatives of stilbene, possessing estrogenic activity, were found capable of inducing tumors in animals.[411a] These observations are especially significant in view of the fact that the chemical configuration of these substances differs essentially from that of natural estrogens, and hence it may be deduced that the biological effect evoked by these substances, and not their chemical nature, primarily determines their carcinogenic activity.

No definite answer can as yet be given to the question of the tumorigenic mechanism of estrogens, but the experimental results enumerated above must inevitably draw attention to the fact that, with very few exceptions, the initiation of neoplastic processes by estrogenic hormones appears to be limited to the female sex organs, the mammary gland and the pituitary, which latter organs are known to exhibit a close func-

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409. J. T. Witherspoon: Am. J. Cancer *24*: 402 (1935).

410. A. Lipschütz & L. Vargas, Jr.: Compt. rend. Soc. de biol. *130*: 9 (1939).

410a. C. F. Geschickter & E. W. Byrnes: Arch. Path. *33*: 334 (1942).

410b. C. D. Haagensen & H. T. Randall: Arch. Path. *33*: 411 (1942).

411. M. B. Shimkin & H. G. Grady: J. Nat. Cancer Inst. *2*: 55; 65 (1941).

411a. E. C. Dodds, W. Lawson & P. C. Williams: Nature, London *148*: 142 (1941).

tional interdependence with the ovary. This difference between the action of estrogenic substances and of carcinogenic hydrocarbons was emphasized at an early time,[412] and the ability of promoting or inducing neoplasia in these organs has been regarded as being closely associated with the normal stimulation exerted by estrogens [364, 413, 414] or has been subordinated to the more general concept of a disturbance of the hormone equilibrium caused by the excessive administration of estrogens.[415, 416] In particular, experimental evidence has been furnished, indicating a synergistic action of the adrenals with estrogens. Following estrenization of mice, the mortality after adrenalectomy was found decreased. On the other hand, the estrogen action was less pronounced in animals in the absence of the adrenals and, accordingly, the tumorigenic effect of estrogens was markedly suppressed in adrenalectomized male mice of a high-cancer strain.[417] Furthermore, it has been stressed that a hereditary susceptibility of animals, or extra-chromosomal factors (milk influence, cf. chapter IX) are necessary requirements governing a tumorigenic effect of estrogens.[418] The genetic factors have been demonstrated as being of an independent nature in regard to mammary cancer or cancers of the uterine cervix in mice subjected to a prolonged estrogen treatment.[419] These complex aspects—interaction with various endocrine mechanisms, dependence on hereditary factors—make it comprehensible that some studies, using varying experimental arrangements, met with failure to induce malignant tumors by means of estrogens,[420, 421] or succeeded only, as quoted above, in producing benign tumors. In particular, it has been reported that estrogen injections into mice during late stages of pregnancy, during lactation and regression, led to a stimulation of the mammary gland, but not to cancer formation, and in this respect no difference was noted between animals of high-cancer or low-cancer strains.[422]

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[412] L. Loeb: Glandular physiology and therapy, A. M. A., Chicago 1935; pp. 177–191.

[413] A. Lacassagne: Am. J. Cancer *28*: 735 (1936); *37*: 414 (1939).

[414] E. Allen: Yale J. Biol. & Med. *10*: 565 (1937–38).

[415] W. Cramer: Am. J. Cancer *30*: 318 (1937); Bull. N. Y. Acad. med. *17*: 3 (1941).

[416] A. Migliavacca: Tumori n.s. *11*: 246 (1937).

[417] W. Cramer & E. S. Horning: Lancet *1*: 192 (1939).

[418] J. J. Bittner: Cancer Research *1*: 290 (1941).

[419] E. Allen & W. U. Gardner: Cancer Research *1*: 359 (1941).

The relations which have formed the subject of the preceding paragraphs have by no means only a theoretical bearing but must also be regarded as of pre-eminently practical significance, on account of the wide-spread use of estrogenic substances in clinical medicine, a form of therapy which will, at present, be considered by the majority of physicians as well-nigh indispensable, not only in the treatment of certain disturbances of the female sex functions, but also of a great number of other pathologies. Accordingly, the crucial question has been asked again and again: "Is it to be feared lest this estrogen therapy may in humans cause the deplorable effect of inducing neoplasia or of enhancing extant malignant tumors?" In general, the answers to this question, based on conscientious deliberations and on careful interpretation of the available results of animal experimentation, have been to the negative.[413, 415] This conclusion has been reached, among other reasons, by taking into account the dosage of estrogens employed and the time required for inducing tumors in animals. It has been calculated that this period of continuous estrogen administration in mice would correspond to seven to ten years in humans.[423] Furthermore, and this is of great significance, very thorough observations of large numbers

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[420] F. Guercio: Tumori n.s. *13*: 412; 477 (1939).

[421] H. Druckrey: Ztschr. f. Krebsforsch. *50*: 27 (1940).

[422] L. Loeb & V. Suntzeff: Cancer Research *1*: 439 (1941).

[423] W. Cramer & W. E. Gye; A. S. Parkes, P. M. F. Bishop & E. C. Dodds: Lancet *2*: 1365 (1936).

of female patients, treated with high estrogen amounts (up to 53,400,000 international units) for long periods of time, did not present any instances of tumor developments which could be charged to this treatment,[382, 424-426] and caution was urged before condemning this valuable therapy.[427] Nevertheless one is absolutely justified in using great care in all instances in which a prolonged medication with estrogenic substances appears indicated, and, in particular, this demand has been made for conditions in which a predisposition to cancer has been suspected to prevail, such as in senile pruritus vulvae.[428] For the same reason, it has been stressed that the indiscriminate use of estrogens, devoid of medical control, may be fraught with great danger, and in this respect attention has been especially drawn to cosmetic preparations containing estrogens, which are promoted commercially and are not subject to medical supervision.[429]

Corpus luteum hormone (progesterone). The interrelationship between this internal secretion of the female gonad and the tumor process has been far less extensively investigated than the hormones of the graafian follicles, and the results reported do not as yet enable the formation of definite conclusions. It has been reported that large numbers of corpora lutea could regularly be found in mice of a strain highly susceptible to spontaneous incidence of mammary cancer. Moreover, when mice of a strain resistant to cancer development were subjected to treatment with estrine ben-

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424. E. Shorr: 3rd Internat. Cancer Congress, Atlantic City, p. 90 (1939).
425. S. H. Geist & U. J. Salmon: Am. J. Obst. & Gynec. *41*: 29 (1941).
426. B. Zondek: Clinical and experimental investigations on the genital functions and their hormonal regulation, Williams & Wilkins, Baltimore 1941.
427. S. H. Geist, R. I. Walter & U. J. Salmon: Am. J. Obst. & Gynec. *42*: 242 (1941).
428. J. A. Schockaert: Bruxelles-méd. *16*: 1667 (1936).
429. J. J. Eller & S. Wolff: J. A. M. A. *114*: 1865 (1940).

zoate and anterior pituitary extracts, an increased formation of corpora lutea was seen in the ovaries of these animals, and in two of them mammary cancers arose.[430] If these findings are interpreted as indicating a participation of the corpus luteum hormone in the etiology of spontaneous mammary cancer in mice, a similar conclusion may also be drawn from another investigation, according to which the parabiotic union of female mice of a high cancer strain with male animals completely inhibited the formation of corpora lutea in the female parabiotic partners and, at the same time, suppressed the development of mammary cancer. Since, moreover, the tumor frequency in breeding females was found markedly higher than in non-breeding ones of the same strain —a fact detailed in chapter IX—it has been assumed that this phenomenon may depend upon the longer persistence of the corpus luteum in pregnancy, thus for a longer time exposing the breeding animals to the supposed tumor-favoring action of the corpus luteum hormone.[431] A more detailed exposition of the latter problem, that is, the influence of pregnancy on neoplastic processes, is presented later. As to additional experimental results, reference is made to the report that progesterone injections stimulated the growth of Flexner-Jobling rat carcinoma,[432] an effect which would agree with the aforementioned observations, but in other studies the growth of animal tumors was not seen to be affected by progesterone [433] or was found to be impeded.[374] The assumption of a tumor-inhibiting property of progesterone even served as the basis for a treatment of human cancer, and the arresting of tumor growth which was claimed to result was considered as being due to a decrease of the

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430. A. Lacassagne: Compt. rend. Soc. de biol. *121*: 607 (1936).
431. W. S. Murray: Am. J. Cancer *30*: 517 (1937).
432. Y. Nitta: Jap. J. Obst. & Gynec. *19*: 512 (1936).
433. A. Picco: Cancro *5*: 134 (1934).

metabolism supposedly caused by this hormone.[434] While neither the theoretical foundation nor the clinical observations reported in this study are convincing enough to establish their validity pending further confirmation, the results of some animal experimentation seem to furnish evidence for the existence of an antagonism between the actions of estrogens and of progesterone, inasmuch as the latter was found to prevent metaplastic changes in the uterine epithelium of animals treated with estrogens.[435, 436] It must, however, be pointed out that proof is lacking as to an ability of progesterone to counteract the effect of estrogenic substances in those instances discussed above, in which the latter was found to be capable of inducing neoplasia.

Pregnancy and neoplasia. Before concluding the discussion of the female gonad in relation to tumor pathology, one cannot omit a brief survey of the relation of pregnancy to neoplastic processes, since the period of gestation—the climax of the female sex function—and its aftermath are known to be accompanied by far-reaching and specific changes of endocrines, involving not only the ovary, but also the pituitary and probably other glands of internal secretion. At the same time, however, significant deviations of the metabolism also take place in pregnancy, and these complex factors—together with some others to be described in detail later—may account for the fact that the observations and experiments did not furnish a clear answer to the influence of pregnancy on malignant tumors. By reviewing a very large literature on clinical experiences, it becomes obvious that the majority of authors interpreted their observations as indicating a stimulation of tumor growth during pregnancy of cancer patients,

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434. P. D. Marinucci: 1st Internat. Cancer Congress, Madrid *1*: 472 (1933).
435. G. Effkemann & L. Herold: Zentralbl. f. Gynäk. *61*: 2865 (1937).
436. V. Korenchevsky & K. Hall: Nature, London *144*: 1048 (1939).

particularly as far as cancers of the genital tract or of the nearby situated rectum are concerned.[437–444] In explaining these findings not so much the endocrine status, but rather the augmented blood supply in the lower abdomen during pregnancy, was thought to be responsible for the enhanced tumor growth. These nutritional factors, however, do not seem to represent the only responsible circumstance, since the formation of metastases has also been seen as being increased[441] and, furthermore, growth stimulation has also been reported of extra-genital tumors, such as of gastric or breast cancers.[367, 445–448] A recent report referred to two cases in which adenomas of the breast had been removed. During subsequent pregnancy of these patients, carcinomas developed in the scars of the previous breast operation.[449] Reference must, however, also be made to a conflicting report, according to which pregnancy retarded the growth of tumors in seventy-two cases, most of them cancers of the breast or of the cervix. The corpus luteum hormone was believed to be responsible for this effect, and a very rapid tumor development, which was observed in those cases after birth or abortion occurred, has been connected with the action of estrogenic substances which are abundantly produced under these conditions.[450]

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437. G. A. Wagner: Monatschr. f. Geburth. *85*: 1 (1930).
438. J. Maršálek; Casop. lék. česk. *68*: 576 (1929).
439. M. Favreau: 1st Internat. Cancer Congress, Madrid *2*: 708 (1933).
440. Levy du Pan: 1st Internat. Cancer Congress, Madrid *2*: 751 (1933).
441. Wagner: Klin. Wchnschr. *8*: 2257 (1929).
442. H. Katz: Arch. f. Gynäk. *143*: 150 (1930).
443. O. v. Franque: Monatschr. f. Krebsbekämpf. *2*: 129 (1934).
444. A. Bromeis: Deutsche Ztschr. f. Chir. *252*: 294 (1939).
445. J. A. Schockaert: Bruxelles-méd. *15*: 1010 (1935).
446. B. J. Lee: Am. J. Obst. & Gynec. *20*: 775 (1930).
447. W. Wachsmuth: Chirurg *5*: 585 (1933).
448. O. H. Rosenthal: Am. J. Surg. *43*: 142 (1939).
449. J. Scapier: Am. J. M. Sc. *202*: 402 (1941).
450. Z. W. Mankin: Arch. f. klin. Chir. *199*: 337 (1940).

The results of studies with animal tumors likewise exhibit an ambiguous character. As to transplanted tumors, some authors failed to notice any influence of pregnancy upon their growth,[451-454] whereas others reported they had observed an inhibition.[455-459] More specifically, it may be stated that a marked inhibition of the growth of Ehrlich carcinoma occurred when the mice were inoculated with a tumor during the second or last third of gestation, whereas tumors transplanted prior to the onset of pregnancy were not affected.[460] An attempt has been made to explain the growth inhibition of transplanted tumors during pregnancy, by assuming that the growing fetus would deprive the tumor of necessary nutritive material [457] and, more specifically, it was believed that a fixation of vitamins by the embryo would impair the growth rate of the tumors, an opinion which seemed supported by the observation of a normal tumor growth in pregnant animals fed an excess of vitamins.[459] Other authors assumed the involvement of endocrine factors dependent on pregnancy, and an experimental basis for such a theory was seen from the isolation of tumor-inhibiting substances from ovaries, placentas and thymus glands of pregnant animals,[461] or from the udders of pregnant cows.[462] While the hormone

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451. I. Kross: J. Cancer Res. *6*: 245 (1922).
452. L. A. Emge & L. M. R. Wulff: West. J. Surg. *42*: 45 (1934).
453. L. A. Emge: Am. J. Obst. & Gynec. *28*: 682 (1934).
454. L. A. Emge, W. Schilling & L. M. R. Wulff: Proc. Soc. Exper. Biol. & Med. *38*: 338 (1938).
455. J. P. Mischtschenko: Ztschr. f. Krebsforsch. *33*: 485 (1931).
456. E. Barbanti-Silva: Ann. di ost. e ginec. *51*: 980 (1929); abstr. Cancer Rev. *5*: 409 (1930).
457. E. v. Graff: Wien. klin. Wchnschr. *27*: 7 (1914).
458. R. Kamekura: Ztschr. f. Immunitätsforsch. u. exper. Ther. *43*: 36 (1925).
459. E. Fränkel & P. Geréb: Ztschr. f. Krebsforsch. *38*: 524 (1933).
460. H. Baatz: Ztschr. f. Geburth. u. Gynäk. *118*: 124 (1938).
461. O. O. Fellner: München. med. Wchnschr. *72*: 676 (1925); Arch. f. Gynäk. *124*: 771 (1933).
462. P. Grabley: Fortschr. d. Med. *50*: 80 (1932).

nature of these substances is very doubtful, a more detailed account on growth-inhibiting factors, derived from placenta or other organs of pregnant animals, will be given in chapter IX (growth factors). The opposite behavior, apparently presented by transplanted animal tumors and by neoplasms in women after the onset of pregnancy is not surprising, since the relations concerned are essentially different. In the first instance a pregnant organism is suddenly affected with neoplasia, in the latter, an organism which has undergone specific pathologic changes, as evidenced by the tumor formation, is gradually transformed in a certain manner during gestation. Actually, the formation of tar cancer in animals, a process which seems to be more comparable to human pathology than inoculation of tumors, has been found stimulated by pregnancy.[463–465] Regarding spontaneous mammary cancers of mice, it has previously been mentioned, and will be discussed in detail in chapter IX, that breeding animals showed a higher tumor incidence than non-breeding ones, but a more recent study has established that pregnancy of the mice only accelerated the appearance of tumors, and that non-breeding animals had the same tumor frequency, though the tumor appearance was delayed.[466] The growth of existent spontaneous mammary cancers of mice was not found to be affected by pregnancy but, post partum and during the start of lactation, a tumor inhibition was frequently observed.[467]

A different problem, rather outside the scope of this treatise, is concerned with the influence of past pregnancies on tumor development and distribution. Statistical analyses

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463. N. Krotkina: Ztschr. f. Krebsforsch. *21*: 450 (1924).

464. S. Ciechanowski & K. Sciesiński: Bull. internat. Acad. pol., Cl. méd. *4*: 141 (1930); abstr. Ztschr. f. Krebsforsch. *35*: Ref. 61 (1932).

465. N. Cini: Fol. gynaec. Genoa *33*: *273* (1936); abstr. Ztschr. f. Krebsforsch. *46*: Ref. 26 (1937).

466. H. B. Andervont & W. J. McEleney: Pub. Health Rep. *53*: *777* (1938).

467. A. Haddow: J. Path. & Bact. *47*: 553 (1938).

have been considered as proving that frequent pregnancies protect women against cancer of the genital tract, while favoring the development of mammary cancer, particularly in women who have lactated.[468] Although these views have not been generally accepted,[469] the author of the former studies sustained his earlier conclusions on the basis of extensive statistical material pertaining to the marital status, pregnancies and cancers of breast and genital organs, and he emphasized, in answering objections raised against this theory, that the relations between pregnancy and cancer in women are entirely different from those in animals.[470]

8. *The Thymus.*

Endocrinology has not as yet been able to dispel the many doubts surrounding the function of this ductless gland, or been able to provide standardized methods for the preparation of its internal secretion, let alone to define its chemical structure. Consequently, studies devoted to the relationship of this organ with neoplasia bear a highly problematic character, and frequently abandon the solid basis of experimental evidence to engage in pure speculation. In this respect, the familiar fact that the time of the greatest activity of the thymus is normally confined to infancy prior to puberty has been mentioned, whereas a physiological involution of the gland occurs with the attainment of sexual maturity, and it was suggested to associate the rare incidence of neoplasia in the prepuberty period with the functional activity of the thymus prevailing during this time but, similarly, as in the case of the gonads, such an argument appears open to objection. Furthermore, it must be remarked that several authors envisaged the physiological role of the thymus as being dependent not so much on its endocrine function, but supposed this organ rather to be a part of the lymphoid system (lymph nodes, spleen, tonsils, etc.).[471] In this connection a concept of far reaching significance has been offered, according to which the thymus and other organs of the lymphoid system are to be regarded as a "cyto-regulatory system," inasmuch as they would decisively determine processes of cell division, and consequently of growth, by storing or making available nucleoproteins, which are very important structural materials of the chromatic cell substance.[472] From another point of view, the thymus has been considered to belong in the enlarged reticulo-endothelial system,[473] and it is obvious that by adopting one of these viewpoints, the interpretation of the role of the thymus in neoplasia will be shifted from the realm of endocrinology to other aspects connected with the systems mentioned.

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[468] S. Peller: Wien. klin. Wchnschr. *35*: 121; 153; 182 (1922); Ztschr. f. Krebsforsch. *21*: 100 (1925); Arch. f. Gynäk. *118*: 59 (1923).

[469] P. Tompkins: Am. J. Cancer *24*: 397 (1935).

[470] S. Peller: Surg., Gynec. & Obst. *71*: 181 (1940).

Morphological findings, and internal secretion. Histological examinations of the thymus, not carried out on clinical material for obvious reasons, but instead on tumor-bearing animals, indicated a marked atrophy of this organ in animals affected with spontaneous or transplanted tumors or with tar cancer.[182, 474, 475] But this finding has not been considered to be primarily connected with the neoplastic process,[474] especially since it was found most pronounced in animals succumbing rapidly to the toxic action of tar application, while it was absent in animals surviving this treatment, with or without developing tumors.[476] However, in rats found resistant to transplantation of Wood's breast carcinoma, the persistence of large thymus glands has been noticed regularly.[477] As to the internal secretion of the thymus, a theory has been advanced according to which an insufficiency of the Hassal's corpuscles, and the lack of their supposed endocrine product, are factors producing a tendency to cancer formation.[478] But in view of completely lacking factual evidence, this conception can be at best considered only as a working hypothesis, the value of which will have to be tested by means of adequate experiments.

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[471] E. V. Cowdry: Special Cytology, ed. 2, Paul Hoeber, Inc., New York 1932.

[472] A. P. Dustin: 1st Internat. Cancer Congress, Madrid *2*: 182 (1933); Cancer, Bruxelles *11*: 25 (1934).

[473] L. Aschoff: Ergebn. d. inn. Med. u. Kinderh. *26*: 119 (1925).

[474] A. Babès: Compt. rend. Soc. de biol. *103*: 165 (1930).

[475] L. Th. Larionow: Ztschr. f. Krebsforsch. *37*: 523 (1932).

Effects of thymus extracts. While, as remarked above, no generally accepted tests are yet available for analyzing and determining the hormone content of thymus extracts, it was reported about thirty years ago that preparations derived from this organ possess a considerable carcinolytic activity, i.e., cause lysis of cancer cells in vitro (cf. chapter X). This phenomenon was thought perhaps to represent one of the reasons why neoplasia is rarely found in infancy, and this view seemed to be supported by the finding of a gradually decreasing carcinolytic power of the blood serum occurring with advancing age, and corresponding to the involution of the thymus gland.[479, 480] However, a therapeutic utilization of this quality of thymus extracts did not yield any beneficial effects. Implantation of calf thymus in cases of inoperable cancer was followed by a moderate and temporary increase of the carcinolytic activity of the serum, but no influence on the tumor growth was noted.[481] Moderate clinical successes were reported to have resulted from the administration of dried thymus gland to patients with inoperable tumors.[482] Somewhat more promising results have been ascribed to a prophylactic implantation of thymus, or injections of thymus extracts, procedures which have been advocated and practiced with cancer patients following radical operations, for the purpose of preventing the recurrence and formation of metastases,[483-485] but doubtlessly an exact evaluation of this therapy requires the careful observation of a much larger clinical material treated in this manner. An extract has been prepared from the "giant epithelial cells of the neck-thymus of young calves," a preparation designated as kankrolysin. Intramuscular injections of this product were reported to have brought about a temporary improvement in four cancer patients, accompanied by softening, necrosis and partial absorption of the tumors.[486] Animal experimentation, however, was unable to disclose any therapeutic effectiveness of the thymus extract (kankrolysin), as tested on spontaneous mouse tumors.[487] Some confirmation for a prophylactic effectiveness of thymus extracts may be implied by the results of animal experimentation with tar cancer. The development of these tumors was found to be impeded by administration of thymus preparations [488] and accelerated in animals subjected to thymectomy.[489] On the other hand, most investiga-

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[476] A. Lamma: Riv. di pat. sper. *8*: 50 (1932); abstr. Ztschr. f. Krebsforsch. *37*: Ref. 57 (1932).

[477] M. Murray: J. Lab. & Clin. Med. *24*: 1247 (1939).

[478] S. Kambosseff: Arch. f. klin. Chir. *188*: 490 (1937).

[479] G. Kaminer & O. Morgenstern: Biochem. Ztschr. *84*: 281 (1917).

[480] E. Freund & G. Kaminer: Biochemische Grundlagen der Karzinomdisposition, Springer, Vienna 1925.

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481. A. Kittinger: Wien. klin. Wchnschr. *42*: 1434 (1920).
482. F. Gwyer: Ann. Surg. *47*: 506 (1908).
483. A. Theilhaber: Wien. klin. Wchnschr. *27*: 206 (1914); Wien. med. Wchnschr. *85*: 1394 (1935).
484. Vercesi (1931); quot. G. Fichera: Chimioterapia del Cancro, Ulrico Hoepli, Milano 1934.
485. W. Karnicki: 1st Internat. Cancer Congress, Madrid *1*: 445; *2*: 46 (1933).
486. M. A. Francois: Cancer, Bruxelles *8*: 1 (1931).
487. W. Karnicki: Ztschr. f. Krebsforsch. *35*: 523 (1932).
488. A. M. Hanson: J. A. M. A. *94*: 653 (1930); Minnesota Med. *13*: 65 (1930).

tions, employing transplanted tumors, yielded inconclusive or negative results, as far as an influence of thymus extracts on tumor growth [490–492] was concerned. Previous implantation of rat or dog thymus into rats inhibited the growth of Wood's breast carcinoma, whereas calf thymus failed to do so.[477] However, the incidence and growth rate of spontaneous mammary cancers in mice was not affected when the animals received injections of thymus extracts.[493]

9. *Alleged Endocrine Factors, and General Theories.*

In addition to the glands with internal secretion discussed so far, several other organs, according to one or another author, are believed to produce endocrine factors which fulfill specific functions, and several investigations have been concerned with hypothetical hormones in connection with problems of tumor pathology. Among such organs, tonsils, placenta and embryonic tissue have formed the subject of a great many studies which supposedly indicated the presence of factors influencing neoplastic growth. But since research has not advanced far enough to present convincing evidence for the true endocrine nature of these substances, or clearly to define their physiological and pathological significance, we refrain from listing these investigations here, and their discussion will take place in connection with other biological growth-inhibiting or growth-promoting factors of hitherto undefined nature (chapter IX). On the other hand, the extensive literature dealing with the effects of spleen extracts, and with the general correlations of this organ with neoplasia, will be surveyed in the section on the reticulo-

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489. B. T. Simpson & M. C. Marsh: Ann. Surg. *93*: 169 (1931).
490. K. Maeda: Tr. Jap. Path. Soc. *20*: 659 (1930).
491. O. O. Meyer & C. C. Simmons: Am. J. Cancer *15*: 2271 (1931).
492. P. Rondoni: Atti acad. med. linċei VI s. *17*: 887 (1933).
493. G. van Smith & E. E. Jones: Proc. Soc. Exper. Biol. & Med. *43*: 157 (1940).

endothelial system (chapter VIII), since it is probable that the reticulo-endothelial elements of the spleen are principally responsible for a variety of interesting phenomena which have been observed.

A concept of broader implications for the cancer problem has called attention to the so-called necrohormones, under which term products of tissue breakdown, liberated from necrotic cells, have been understood. It has been believed that these substances, which seem also to be involved in the processes of physiological regeneration, represent growth factors. They may start the formation of malignant tumors when accumulated in sufficient concentrations in receptive tissues, the vitality of which is impaired in consequence of inadequate oxygen supply. On the other hand, the necrohormones have been regarded as potent stimuli for the functional activity of the reticulo-endothelial system, and such an effect has been believed to represent an unspecific common factor underlying a great variety of inorganic, organic, or immunologic therapeutic measures. Inasmuch as all these interferences result in tissue destruction, and the necrohormones, consequently formed, were held to be responsible in an indirect manner for the tumor inhibition observed, i.e., by activation of the reticulo-endothelial function.[494] Other authors attributed a particular significance to the finding of local lymph stasis in tissues exhibiting precancerous lesions, on the assumption that the impaired circulation would prevent the supply of the tissues with certain tumor-inhibiting endocrine factors, which were not specified.[495] If a predominantly hypothetical nature must be assigned to these viewpoints, this appears to be even more the case in regard to other theories, which assumed an essential connection to exist between insufficiencies or disturbances of specific en-

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494. W. Caspari: Arch. f. klin. Chir. *146*: 711 (1927).
495. W. S. Handley: Brit. M. J. *2*: 607 (1929).

docrine glands and cancer etiology,[81, 247, 496–498] or even envisaged the causative tumor agent as being a hypothetical "hormone" of the neoplasms.[499] In complete contrast to such opinions, other authors, by interpreting the available experimental data, denied the existence of convincing proof for a primary involvement of the endocrine system in the tumor process.[500, 501] Another research worker came to similar conclusions in an investigation of glands with internal secretions of mice subjected to the carcinogenic stimulus of benzpyrene. Some alterations found in endocrine organs were regarded as being merely secondary phenomena, but the author did not feel justified in excluding the possibility of an involvement of endocrines in the spontaneous tumor formation.[502] The effects of a deficiency or of an excess of hormones on carcinogenesis was recently studied on a broad basis. Rats, injected subcutaneously with methylcholanthrene, were subjected to removal of various endocrines, whereas other groups of animals received injections of hormones. As compared with control animals, these interferences did not appreciably influence the tumor induction, with the following exceptions. A statistically significant deviation from the response of control animals was observed in rats to which gonadotropic hormones were administered—acceleration of tumor development—whereas injections of adrenal cortex extract delayed the tumor induction.[503] The authors of this study stressed that these results may not be entirely conclusive, because the

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[496] J. Paulsen: Ztschr. f. Krebsforsch. *21*: 119 (1924).

[497] H. Elsner: Deutsche med. Wchnschr. *50*: 1349; 1391 (1924); Krebsentstehung und endokrines System, Karger, Berlin 1926.

[498] J. Samuels: Jap. J. Obst. & Gynec. *22*: 132–189 (1939).

[499] P. Zadik: Acta Cancrol. *1*: 37 (1934).

[500] O. M. Gruhzit: Ann. Int. Med. *4*: 1589 (1931).

[501] B. Fischer-Wasels: Endokrinologie *14*: 100 (1934).

[502] L. Th. Larionow: Am. J. Cancer *38*: 492 (1940).

[503] D. L. Smith, J. A. Wells & F. E. D'Amour: Cancer Research *2*: 40 (1942).

dose of the carcinogen employed—leading to 100 per cent tumor development in control animals—may have been too high to permit some of the effects of the interferences with endocrines to become manifest. For this reason, continued study of the interaction of endocrines and carcinogens may be desirable.

Summary.

Although no attempt is made to offer predictions as to future results from endocrinological cancer research, from the large material available on endocrines in tumor pathology it may be safely concluded that it is not possible to regard neoplastic diseases as being primarily caused by the insufficiency, or overfunction, of one or another gland with internal secretion, nor does it seem likely that continued investigations may establish such an etiology. When, on the other hand, in the preceding survey there was occasion to make reference to a number of morphological and functional alterations of the endocrines, which had convincingly been proven to occur in malignancy, it has been emphasized by most of the authors of these reports that they regarded these findings merely as secondary consequences of the tumor process. According to the writers' belief, it would be even more astounding if the organs of internal secretion in tumor-bearing hosts had not been shown to be affected, since it is common knowledge that all severe diseases lead to disturbances of endocrines in one or another manner. Bearing in mind this unspecific nature of endocrine alterations in neoplasia, it will be easily understood that a diagnostic utilization of hormone determinations can hardly meet with success, excepting in those instances of malignant tumors which arise from an endocrine organ itself and which retain their characteristic functional activity (chorionepithelioma, certain tumors of the ovary, adrenals, or pancreas, etc.).

Yet a different aspect has been revealed by a large series of interesting investigations performed during the last decade, in which a participation of hormones in the development of certain malignant tumors has been demonstrated, that is, in the case of estrogenic substances. The fact may again be stressed here, viz., that the effect of these hormones has been recognized as being definitely distinguishable from the action of other carcinogenic stimuli, inasmuch as estrogens have been found to elicit tumor formation almost exclusively in those organs which normally are subject to their regulating influence, whereas, for instance, carcinogenic hydrocarbons, or other organic carcinogens, are effective in a great variety of tissues and organs. In this respect, a theory of perhaps far reaching significance has been proposed, i.e., it was believed probable that, in a manner similar to the estrogens, other hormones when administered in excessive amounts may also provoke neoplasia in organs for which they constitute a physiological stimulus,[860] and experimental work, based on such a concept, unquestionably should produce interesting results. In several studies, mentioned above, attempts have been made to explain the action of an oversupply of certain hormones (estrogens) in those instances where tumor formation results, by assuming that this phenomenon is due to specific disturbances in the hormone equilibrium, and a support for this view has been seen in findings which indicated the presence of analogous alterations of the endocrine balance in animals of inbred strains exhibiting an increased susceptibility to spontaneous neoplasia. Although further confirmation of these observations is required before their final acceptance, and although human tumor pathology unquestionably exhibits much more complex relations as to hereditary and environmental factors, the progress of endocrinology may yet lead to a point where it would be possible to ascertain at an early time the existence of such disturbances in the hormone equilibrium of humans; when, furthermore, methods are devised for restoring these abnormal relations, these will represent a valuable contribution for the prevention of certain types of malignant tumors, though not of neoplasia in general.

While it is conceded that the realization of such a prophylactic measure for the purpose of combatting cancer is by no means a simple matter, an entirely different problem—and in the writers' opinion exhibiting still less promising aspects—is represented by the therapeutic use of endocrines when tumor development has already taken place. It would, however, not appear justified a priori to reject an endocrine cancer therapy on account of the failure to establish an etiological relationship between neoplasia and glands with internal secretion, for, in addition to their physiological function, many hormones also possess pharmacological qualities, and consequently have proven to be a valuable means of treatment in diseases not causally related to the endocrines. Yet as far as the use of hormones for the treatment of malignant tumors is concerned, clinical experiences, at least collected so far, have not been capable of sustaining the validity of a host of theories which ascribed to various hormones a tumor-inhibiting property, and this notwithstanding numerous reports indicating such an effect as having been caused in animal experiments. Among these, a great number have been intended to achieve this aim by making use of two qualities inherent in certain hormones. One, the regulation or inhibition of growth in general; two, the interference with metabolic processes in a manner which may prove detrimental for the vitality of tumors. When it has not been possible to derive practical benefits from such procedures—a fact which is evidenced by the irregularity of the results obtained even in animal experiments which also led to numerous conflicting statements—one of the reasons responsible for this failure may be seen in the fact that neoplasms, in accordance with their quality of "autonomous" growth, are much less subject to the regulatory influence of hormones than normal tissues. Hence, it would seem more promising to employ an endocrine therapy principally following surgical removal of the tumors as a supporting measure for preventing recurrence and spread of metastases, insofar as treatment with hormones may appear at all justified, on the basis of continued experimental work.

CHAPTER VIII

IMMUNOLOGY

THE presentation of this chapter is essentially different from that of the preceding ones, inasmuch as the subject in question can be sketched only in rough outlines. This is necessitated by the fact that immunology for the greatest part must be regarded as lying outside the limits of biochemistry and, accordingly, a critical attitude might even raise objections against the inclusion of this topic in a book dealing with the chemistry of malignant tumors. On the other hand, it seems to the authors that the borderlines are even today somewhat indistinct and may become more so with advancing knowledge. Endocrines, vitamins and serologic tumor diagnostics represent subjects from which definite connections already lead to the field of immunology. Certainly the interior of the strange realm is more or less impervious to chemical research but in the same way as unexplored parts of the earth are charted in maps, at least in their outlines, it is felt appropriate for us to draft the principal features of this only insufficiently clarified branch of cancer research.

Apart from tumor pathology, immunology is inseparably associated with the vast and very interesting part of science which is based on antigen-antibody reactions, and which has resulted in valuable accomplishments as to the theoretical insight into, and the practical treatment of, a great many diseases. When expectations of this kind are transferred to immunology of cancer, they will be inevitably disappointed. For, in the strict sense of the word, immunity—according to the classical definition—can be spoken of only in those instances in which a pathology is due to a specific causative factor (of living or inanimate nature), and against which the body is capable of forming specific antibodies. But in the case of malignant tumors such an assumption is—according to present opinion—not acceptable to the majority of cancer research workers. That is, in general, neoplasia cannot be regarded as an infectious disease due to a certain pathogenic micro-organism. Those instances of animal tumors in which an infectious etiology (virus) has been established beyond all doubt will be dealt with in the next chapter, but the prevailing opinion tends to regard those and other micro-organisms which give rise to malignant tumors, not as the specific "cancer-germ," but as one of the numerous stimuli which are known to induce the formation of neoplasms. Hence, by denying the infectious nature of neoplasia it would seem that the support is withdrawn from the application of immunological aspects, in the strict sense of the word, to tumor pathology. Yet, in spite of this fact other trends of thought have called attention to phenomena which might establish a basis for an immunology of cancer in a broader sense. In this connection it was supposed that the deviation of the tumor cell from the normal body cell may reach such an extent that a formation of antibodies could be suspected, and this is an extremely difficult problem which has formed the subject of a great many investigations dealing with specific tumor antigens and tumor antibodies. But prior to the discussion of these fundamental questions pertaining to tumor immunology, it will be necessary first to turn to another group of phenomena which for the longest time had attracted the interest of oncologists, because of an ostensible resemblance to immunological factors. This refers to the problems of disposition or of resistance to neoplasia. In an analogy to infectious diseases it was presumed that it might be possible to interpret different reactivity, expressed in a variable susceptibility to the development and growth of tumors, with the nomenclature of immunology, and accordingly the terms of "natural immunity" and of "acquired immunity"—the latter subdivided into "active" and "passive immunity"—have been applied to these phenomena. It is obvious that the decision as to whether or not this interpretation is justified will hinge entirely on the definite proof of tumor-specific immune bodies, a subject discussed in the second part of this chapter. Notwithstanding the fact that these problems have not been answered satisfactorily, so far, a great many authors advanced one step further in their work, and tried to avoid the theoretical impasse by means of experiments aiming at demonstrating an effectiveness of immunotherapy in malignancy. These studies will be detailed in the third and fourth parts of this chapter which deal with specific or unspecific immunotherapy of neoplasia. Finally, a separate presentation has been reserved in the last part of this chapter for the relationship between malignant tumors and the reticulo-endothelial system, since this system, in general, has been recognized as constituting an important factor in immunological processes and, in particular, has been found, on the basis of many observations and experimental findings, to exhibit a close interdependence with neoplasia.

1. *Disposition and Resistance to Neoplasia.*

Both clinical observations and findings of animal experimentation have strongly impressed cancer research workers with the fact that certain individuals exhibit a striking resistance against the formation of malignant tumors, and others a striking susceptibility when exposed to the same stimulus. In human pathology, this is illustrated by the instances of occupational cancers, and numerous animal experiments have led to similar conclusions. For example, the different reactivity of various species may be mentioned, e.g., mice respond to application of tar very regularly with cancer formation, whereas rats are highly resistant. But, also, within one susceptible species remarkable differences become apparent, as expressed in the different lengths of the latent period and the different rate of tumor advancement, which may be observed in single individuals of the same species. However, certain stimuli appear to be so effective that carcinogenesis results even in comparatively refractory species or animals, whereas other weaker stimuli require the presence of a special disposition to neoplasia in the animal exposed to it. While no attempt at mathematical accuracy is intended, these relations may be illustrated to some extent by a hypothetical formula, according to which probability of carcinogenesis depends on the product of strength of stimulus and of disposition to neoplasia. We cannot omit mentioning that, apart from immunological concepts, a great many other theories have been advanced with the purpose of defining more clearly the nature of disposition or resistance to cancer. In this connection, the theory of carcinolytic and antilytic factors of Freund and Kaminer,[1] the alkalosis theory of Reding,[2] the metabolism theory of Warburg [3] and of Fischer-Wasels,[4] the theory of an involvement of the reticulo-endothelial system according to Braunstein [5] and Fichera [6] may be recalled. Although these and several other principles have been discussed and evaluated in other chapters of this book, it may be stated here that their significance seems to lie less in their established validity, rather than in the fact that they serve as working hypotheses for studies performed by the respective authors.

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[1] E. Freund & G. Kaminer: Biochemische Grundlagen der Karzinomdisposition, Springer, Vienna 1925.

[2] R. Reding: Le terrain cancereux and cancérisable, Masson, Paris 1932.

[3] O. Warburg: Über den Stoffwechsel der Tumoren, Springer, Berlin 1926.

[4] B. Fischer-Wasels: Gastherapie bösartiger Geschwülste, Frankfurt a. M. 1929.

[5] A. Braunstein: Ztschr. f. Krebsforsch. *32*: 118 (1930).

[6] G. Fichera: Chimioterapia del cancro, Hoepli, Milano 1934.

Natural immunity. This phenomenon has been known almost from the very commencement of experimental cancer research;—that is, since work with inoculated tumors was initiated. The most striking example in this regard is represented by the category of heterologous immunity, i.e., by the failure of successful heterotransplantation. The oldest explanation for the impossibility of transplanting tumors of one species into another was given by Ehrlich[7] in the term "athreptic" immunity. He assumed that every neoplasm requires for its growth a specific nutritive material, which can be provided only by certain hosts, and he saw a proof for the validity of this supposition in the results of the so-called "cross-transplantation." A mouse tumor, when inoculated into a rat, may persist for a short time, that is for as long as the nutritive substances contained in it can support growth, but after such time the tumor regresses unless it is retransplanted to a mouse. In this animal, the growth intensity recovers but decreases again rapidly when a renewed transfer to a rat is performed. Another theory[8, 9] held a lymphocytic reaction occurring in the host inoculated with tumor tissue foreign to the species to be responsible for the failure of the transplant to grow. The local round-cell infiltration was demonstrated to represent a defensive mechanism occurring following grafts of foreign tissues, and hence this phenomenon must be subordinated under the general law which states that heterologous transplants of any tissue cannot persist. More recently the hypothesis of athreptic immunity has been revived in a more modern form, inasmuch as the nutritive material necessary for the tumor growth was held to be identical with vitamins; that is, successful heterotransplantation was reported to be possible when the hosts of a foreign species were fed an excess of vitamins.[10] However, it seems extremely doubtful whether these findings can be generalized; that is, whether vitamins represent the decisive factor for the outcome of heterotransplantation, since other methods, not dependent on nutrition, have also been described as being capable of facilitating heterologous tumor growth, the methods being, for instance, impairment of the reticulo-endothelial function,[11, 12] parenteral administration of proteins foreign to the species,[13, 14] or inoculation into embryonic or nervous tissue.[15] Temporary, but quite intensive growth of Crocker mouse sarcoma was observed in rats when the tumor was transplanted to hosts which had received a preceding roentgen irradiation.[16] In addition, some authors devoted extensive studies to certain heterologous tumor strains in rats which were derived from a human neoplasm [17] or from the Ehrlich mouse carcinoma.[18] However, the latter, known as "Ehrlich-Putnoky rat carcinoma" has been declared to represent merely a temporary growth of mouse tissue in the rat, endowed with a strong proliferative capacity, and, in general, the value of studies with heterologous tumor transplants has been denied.[19] In contrast to this view a summary, given by Putnoky on the results of seven years of experimentation with the Ehrlich-Putnoky carcinoma, stressed the fact that this tumor, although foreign to the rat species, exhibited characteristic qualities distinguishing it from other rat tumors and mouse tumors as well, and that significant information may be gained from these investigations, pertaining to the immunological reactivity of animals.[20]

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[7] P. Ehrlich: Ztschr. f. Krebsforsch. *5*: 59 (1907); Verhandl. d. deutsch. path. Gesellsch., Kiel 1908.

[8] J. B. Murphy & E. Sturm: J. Exper. Med. *42*: 155 (1925).

[9] J. B. Murphy: The lymphocyte in resistance to tissue grafting, malignant diseases and tuberculous infection; Monographs Rockefeller Inst. M. Research No. 21 (1926).

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[10] E. Fränkel & P. Geréb: Ztschr. f. Krebsforsch. *38*: 524 (1933); *39*: 93; 97; 101 (1933).

[11] B. E. Brüda & H. Pfeiffer: Ztschr. f. d. ges. exper. Med. *68*: 116 (1929).

[12] G. Roskin: Ztschr. f. Krebsforsch. *24*: 122 (1927).

[13] H. Auler & K. Pelczar: Ztschr. f. Krebsforsch. *27*: 104 (1928).

[14] F. P. Tinozzi: 1st Internat. Cancer Congress, Madrid *1*: 87 (1933).

[15] C. Lewin: Ergebn. d. inn. Med. u. Kinderh. *1*: 157 (1908).

[16] J. Clemmesen: Am. J. Cancer *29*: 313 (1937).

[17] A. Kritschewski & S. Sinelnikow; quot. I. L. Kritschewski & P. L. Rubinstein: Ztschr. f. Krebsforsch. *28*: 96 (1928).

[18] J. Putnoky: Ztschr. f. Krebsforsch. *32*: 520 (1930); *39*: 451 (1933).

An important factor relating to disposition and resistance to inoculation of tumors is represented by the different susceptibility exhibited by different animal strains of the same species. This was demonstrated long ago for the Ehrlich mouse carcinoma, by the fact that in mouse strains of different origin the success of transplantation varied widely.[21] This phenomenon is still more pronounced as far as spontaneous tumor development is concerned; in this case, both genetic and extra-chromosomal factors have been recognized as playing a decisive role, relations which will be discussed in detail in chapter IX. But also regarding the response to chemical and physico-chemical stimuli, capable of inducing neoplasia, significant distinctions have been established as existing between different animal species and animal strains, which facts are deserving of the greatest consideration for the exact performance and proper evaluation of experiments. Even beyond the special qualities of various strains, individual animals of the same strain have been found to be distinguished from one another by their susceptibility to malignant tumors; numerous observations have demonstrated the fact—which may be significant for human pathology—that a certain number of animals fail to respond to transplantation or to carcinogenic substances with tumor growth (refractory animals). This individual resistance has been studied with regard to the Ehrlich mouse carcinoma, later also regarding the Rous sarcoma [22] and the Brown-Pearce rabbit carcinoma,[23] and is observed more or less with most inoculated or chemically induced tumors. As to the interpretation of this phenomenon, two opinions have been expressed. One envisages an unspecific resistance determined by metabolic, enzymatic, or endocrine factors, the other assumes the presence of a true immunity. In support of the first view, investigations may be mentioned according to which rats, refractory against tumor transplantation, were found to exhibit a characteristic deviation from the normal nitrogen metabolism. Following a single roentgen irradiation of these animals, a considerable drop of the blood urea concentration was noted, an effect which failed to occur in tumor-susceptible animals.[24, 25] Similar findings have also been obtained more recently in animals experimentally immunized against tumor inoculation,[26] but beyond the general assumption of a metabolic-enzymatic alteration, no specific explanation can as yet be offered for this phenomenon. A different position must be assigned to the experiments of Freund and his collaborators [27, 28] who succeeded in varying the susceptibility of animals to tumor transplants by means of changing the character of their intestinal flora. The theory which initiated these studies concerning the presence of carcinophil or carcinophobe intestinal bacteria as an essential factor for the disposition to neoplasia will be presented in chapter X. In regard to the other alternative ascribing a genuine immunity to tumor-refractory animals, two viewpoints appear possible, referring to either a humoral or a cellular immunity. The first viewpoint may justifiably be applied to the Rous sarcoma since it was observed that the serum of refractory animals is also capable of neutralizing the Rous sarcoma agent in vitro.[29] It must, however, be stressed that the immunological aspects apparent in Rous sarcoma and other virus tumors cannot simply be transferred to other types of neoplasia and, accordingly, these relations will be discussed in detail in the third part of chapter IX. Yet, in this connection, a report must be brought to the attention of the reader, according to which rat carcinoma explants failed to grow when plasma of rats, resistant against inoculation of this tumor, was used as the culture medium, an inhibition which was found to be tumor-specific.[30] As to the assumption of a cellular immunity, no general agreement has been reached; no proof for the existence of such a phenomenon was furnished in a study in which tumor tissue explants were cultivated together with tissue derived from normal organs of tumor-resistant animals, inasmuch as the growth of the tumor tissue was not affected by this procedure.[31] These findings are contrasted with the results of another author who found in neoplastic tissue cultures a considerable growth inhibition when tissues of animals artificially immunized against this tumor were added.[32] In connection with cellular immunity, reference may be made to the existence of a kind of "local immunity" or "local disposition," expressed by the fact that some tumors grow best or exclusively when inoculated into a certain tissue, e.g., the Brown-Pearce carcinoma in the testis of rabbits. In other tumors only quantitative differences are apparent, according to the site of the transplantation; that is, variable yields of tumors and different growth rates are observed when subcutaneous, intramuscular or intraperitoneal inoculations are performed. One will hardly go amiss in assuming that these divergencies depend on different nutritive conditions encountered by the tumors in the respective tissues, a behavior perhaps related to the concept of Ehrlich regarding athreptic immunity. Recently evidence was presented indicating the existence of a sex difference in mice as to their susceptibility or resistance to transplanted tumors. Following inoculation of sarcoma 37, male mice exhibited a greater percentage of takes, and less spontaneous regressions than female animals. Furthermore, the procedure of "intradermal immunization" (cf. p. 654) resulted in a greater resistance of female than of male animals: tumor growth was observed in 11 out of 42 "immunized" male mice, but only in 4 out of 132 female mice.[32a]

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[19] F. R. Selbie: Am. J. Cancer *28*: 530 (1936).
[20] J. Putnoky: Am. J. Cancer *32*: 35 (1938).
[21] L. Michaelis: Deutsche med. Wchnschr. *33*: 826 (1907).

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[22] A. Fischer: Ztschr. f. Krebsforsch. *24*: 580 (1926).

[23] W. A. Collier & A. Cohn: Ztschr. f. Krebsforsch. *35*: 641 (1932).

[24] E. C. Dodds, W. Lawson & J. C. Mottram: Biochem. J. *19*: 750 (1925).

[25] E. C. Dodds: Am. J. Cancer *15*: 2765 (1931).

[26] R. H. Oster & W. T. Salter: Am. J. Cancer *32*: 422 (1938).

[27] E. Freund, B. Lustig & B. Kellner: Ztschr. f. Krebsforsch. *37*: 355 (1932).

[28] E. Freund & B. Lustig: Wien. klin. Wchnschr. *46*: 1580 (1933).

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[29] F. G. Banting & S. Gairns: Canad. M. A. J. *30*: 615 (1934).

[30] E. Rerrich & V. Wettstein: Frankfurt. Ztschr. f. Path. *48*: 353 (1935).

[31] A. L. Wylegschanin: Ztschr. f. Krebsforsch. *38*: 99 (1932).

[32] E. Centanni: Ztschr. f. Krebsforsch. *28*: 47 (1929).

Acquired, active immunity. In the following, endeavors made during almost four decades by numerous authors who sought to establish proof of the fact that the susceptibility of animals to neoplasia may be decisively changed by a preceding treatment with tumor material, especially as far as transplantable tumors are concerned, will be surveyed. It is obvious that the premise of such undertakings must be seen in the assumption that tumors contain more or less specific antigens, the introduction of which into the body would give rise to the formation of counteracting antibodies and, accordingly, the effects of such a treatment, if demonstrable, would correspond to the phenomenon of acquired active immunity apparent in a great many infectious diseases. Whether or not this premise is valid appears to be very problematic at present, in view of the inconclusive results of studies devoted to tumor-specific immune bodies which are discussed in the next part of this chapter. Furthermore, it will be shown later that in many investigations entirely unspecific antigens have been employed for the purpose of "immunizing" animals against tumor development and, consequently, in these instances, the term of "immunity" in a classical sense of the word does not appear applicable to the effects observed. Since, as stated in the introduction, this presentation is not intended to enter into a complete enumeration of all studies which have been performed, the extensive literature of older date is not discussed in detail, but reference is made to several reviews which have summarized this work.[33-36, 36a] The fundamental conclusion that may be drawn from these investigations may be expressed in the fact that the chance of producing immunity in animal experiments by means of the administration of tumor material varies directly with the virulence of the neoplastic tissue employed. On the other hand, all those experiments in which the production of immunity was attempted by using damaged or attenuated tumor tissue (formol,[37]

[32a] L. Gross: Proc. Soc. Exper. Biol. & Med. *47*: 273 (1941); Cancer Research *1*: 880 (1941).

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33. H. Apolant: Ztschr. f. Immunitätsforsch. u. exper. Ther. *10*: 103; 159 (1911); in W. Kolle & A. v. Wassermann: Handbuch der pathogenen Mikroorganismen, Fischer, Jena 1913, Vol. *3*: 167.

34. Uhlenhuth & Seiffert: Med. Klin. *21*: 376; 616 (1925).

35. W. Caspari, A. Kolle, R. Kraus & P. Uhlenhuth: Handbuch der pathogenen Mikroorganismen, Fischer, Jena 1929, Vol. *1*: 1225.

36. W. H. Woglom: Cancer Rev. *4*: 129 (1929).

36a. R. R. Spencer: J. Nat. Cancer Inst. *2*: 317 (1942).

37. G. Tizzoni & G. de Angelis: Ann. igiena *40*: 354 (1930); Atti Acad. Naz. Lincei *11*: 645 (1930); abstr. Ztschr. f. Krebsforsch. *33*: Ref. 194 (1931).

chloroform,[38] heat,[39, 40] reduction of doses [41]) met with a very limited or complete lack of success. In interpreting these findings it may be thought either that only very virulent material stimulates the hypothetical defensive mechanisms of the body, or—and this seems more probable—that the so-called active immunity in reality represents but a quantitative increase of already existent defensive forces, an effect which can be achieved only by large doses of the "antigens" employed. Several special instances of experimental immunity have been described, among which Ehrlich [7] called especial attention to the fact that a second tumor inoculation into an animal bearing a transplanted tumor as a rule fails to result in tumor growth. Whereas this author explained his finding according to his concept of athreptic immunity—that is, he assumed that the first tumor would deprive the second of the necessary nutritive material—Woglom [36] attached the term of "concomitant" immunity to the phenomenon and showed that it was by no means constant, but instead depended on the type of tumor and on the time intervals between the first and second inoculation. Further analyses of concomitant immunity have been performed by other authors who arrived at similar conclusions.[42–44]

Another group of studies has dealt with the so-called "operative" immunity; that is, it was found that radical extirpation of the tumor was followed by resistance of the animal to renewed inoculation of this tumor, whereas this effect failed to take place when the tumor had not been re-

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38. K. Nather: Ztschr. f. Krebsforsch. *19*: 115 (1922).
39. L. Kepinow: Compt. rend. Soc. de biol. *83*: 785 (1920).
40. K. Sugiura & S. R. Benedict: Am. J. Cancer *15*: *2727* (1931).
41. K. Nather & H. Schnitzler: Wien. klin Wchnschr. *39*: 1384 (1926).
42. F. D. Bullock & G. L. Rohdenburg: J. Cancer Res. *5*: 119; 129 (1920).
43. H. B. Andervont: Pub. Health Rep. *47*: 1859 (1932).
44. J. J. Bittner: Am. J. Cancer *28*; 127 (1936).

moved completely or when recurrence set in.[38] The general reproducibility of these results has been contested,[45] but positive findings have been obtained in special instances. Tumors inoculated into the tails of mice, when extirpated, produced immunity of the animals,[43] and similar results were obtained with Ehrlich mouse carcinoma transplanted subcutaneously, but significantly not with spontaneous mouse carcinomas.[46] Particular interest may be assigned to those investigations in which the immunological relations were studied with animals in which spontaneous regression of an inoculated tumor had taken place, and the animals proved resistant against subsequent transplantation. The question arose whether this apparent immunity was caused by the resorption of the first tumor, or whether it had existed prior to this event. A number of older studies decided, by means of various experimental arrangements, in favor of the second alternative.[47-50] Continued work in the same line demonstrated by means of histological examinations that the regression of the second tumor was accompanied by a peripheral necrosis, and by infiltration of connective tissue, and that these effects were due to a "retarding agent" probably originating in the spleen.[51] Very interesting experiments have been reported in which the nature of this retarding agent was analyzed; rats in which inoculated sarcomas had receded spontaneously were subjected to a second inoculation of this sarcoma, and the subcutaneously injected tumor suspension was removed after four days. Since it could be assumed that this tumor material had adsorbed the retarding agent, it was extracted with Locke solution, the extracts were incubated with fresh sarcoma suspensions for two to six hours, and this material was inoculated into rats. By comparing the growth of the resulting tumors with adequate control tumors a marked growth inhibition or complete failure of tumor formation was observed; an adsorbability of the retarding agent to charcoal was demonstrated, but curative experiments with this substance led to inconclusive results because of its high toxicity.[52] These highly significant findings make further investigation desirable, yet they are complicated by the difficult experimental conditions. A resemblance to these findings may be seen in a report, according to which a special tumor immunity was produced in mice in the following manner. A mouse sarcoma was transplanted to rabbits, removed after a few hours and inoculated into mice; no tumor formation resulted and animals thus treated, when re-inoculated after eleven days with this sarcoma, showed only fifty-five per cent of tumor takes, whereas in controls eighty to one hundred per cent positive transplantation results were obtained.[53] It has been repeatedly stressed that the immunity resulting from the regression of a previously transplanted tumor is generally restricted to the same type of tumor, and does not extend to other neoplasms. These relations have been confirmed in experiments of recent date, according to which the regression of Jensen sarcomas or Flexner-Jobling carcinoma protected rats only against the same type of tumor but not against the other; the protective action was found the greater, the larger the size the first tumor had reached prior to its disappearance,[54, 55] a finding which does not seem in complete accord with the results of earlier work.[47-50] However, most recently, the significance of acquired tumor-resistance, as exhibited in the experiments mentioned, has been greatly reduced in its specific nature, since it was assumed that these phenomena depend entirely on genetic differences inherent in the inoculated animal on the one hand, and the animal in which the tumor originally was produced on the other. Proof of this view was furnished by the fact that embryonic skin, derived from mice of an inbred strain, did not immunize against tumors of the same line, but was active when administered to animals of a partially related line.[56]

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[45] E. Ebeling: Ztschr. f. Krebsforsch. *16*: 121 (1917).

[46] Ch. Hackmann: Ztschr. f. Krebsforsch. *50*: 352 (1940).

[47] B. R. G. Russell: 5th Sc. Rep. Imp. Cancer Res. Fund, London, p. 1 (1912).

[48] M. Haaland: Norsk Mag. f. Laegevidensk *7*: 1047 (1909).

[49] J. C. Mottram & S. Russ: Proc. Roy. Soc., London s. B *90*: 1 (1917).

[50] W. H. Woglom: J. Cancer Res. *9*: 171 (1925).

[51] W. H. Woglom: Am. J. Cancer *17*: 873 (1933).

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[52] W. H. Woglom: Am. J. Cancer *30*: 477 (1937).

[53] R. Fischer & H. Luschinsky: Compt. rend. Soc. de biol. *122*: 764 (1936).

[54] G. Klein & J. Klinke: Ztschr. f. Krebsforsch. *44*: 240 (1936).

[55] J. Klinke: Ztschr. f. Krebsforsch. *46*: 1 (1937).

A different approach for producing tumor immunity has been chosen in the form of intracutaneous inoculation, which procedure was found to be effective by some investigators,[57, 58] while others contested this claim.[59, 60] A great deal of work has been devoted to this question within the last years by Besredka, Gross and their collaborators.[61, 62] It was first reported that intracutaneous inoculation of Brown-Pearce carcinoma into rabbits left the animals, following regression of these tumors, immune against intravenous transmission of the same tumor,[61, 63] and later these animals were also found to be refractory against intratesticular tumor transplantation.[64] It was assumed that the resorption of the intracutaneous tumors, which regularly took place, caused a true

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56. M. J. Eisen & W. H. Woglom: Cancer Research *1*: 629 (1941).
57. E. Knopf: Ztschr. f. Krebsforsch. *25*: 64 (1927).
58. A. Lafranchi, G. G. Palmieri & E. Seren: Nuova vet. *15*: 1 (1936); abstr. Ztschr. f. Krebsforsch. *45*: Ref. 316 (1937).
59. E. Urbach & H. Schnitzler: Wien. klin. Wchnschr. *41*: 941 (1928).
60. E. Urbach & A. Wiedmann: Med. Klin. *29*: 742 (1933).
61. A. Besredka, I. Magat & P. Besnard: Compt. rend. Acad. d. sc. *201*: 170 (1935).
62. L. Gross: Am. J. Cancer *31*: 609 (1937).
63. A. Besredka & M. Bardach: Compt. rend. Acad. d. sc. *202*: 2193 (1936).
64. A. Besredka: Cancer, Bruxelles *12*: 115 (1935).

and lasting immunity of the animals,[65] and the specific character of this effect should be proved by experiments in which such an immunity failed to occur in animals subjected to intracutaneous inoculation of normal tissues.[66] Furthermore, this immunity was recognized as being of cellular nature[66] since the immunity could not be transferred to parabiotic animals of which one partner had acquired the immunity.[67] In later work the intracutaneous immunization of rabbits was observed also to protect the animals against transplantation of Brown-Pearce carcinomas into the stomach,[68] and an immunity of similar character was produced in fowl by intracutaneous injection of small amounts of Rous sarcoma; in fifty per cent of the animals thus treated, stationary tumors developed which were later resorbed and left the animals resistant against renewed Rous sarcoma transmission. In this case, too, the immunity was found to be of a local nature and not transferable.[69] A number of authors, however, were unable to confirm these findings; that is, no immunity against Brown-Pearce carcinoma was observed following intracutaneous inoculation of tumor material.[70-73] In one of these studies, metastases were even observed in fifteen per cent of the animals which had been inoculated only intracutaneously,[70] and other authors supposed that the divergent results of Besredka might have been due to infection of the tumor tissue used by him.[72] On the other hand, several reports have

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65. A. Besredka, I. Magat, P. Laval & P. Besnard: Ann. Inst. Pasteur *56*: 125 (1936).
66. A. Besredka & L. Gròss: Ann. Inst. Pasteur *57*: 342 (1936).
67. A. Besredka & L. Gross: Ann. Inst. Pasteur *60*: 5 (1938).
68. A. Besredka & L. Gross: Ann. Inst. Pasteur *62*: 253 (1939).
69. A. Besredka & L. Gross: Ann. Inst. Pasteur *60*: 465 (1938).
70. Y. Nozu: Acta dermat. *26*: 66 (1933).
71. J. Flaks & B. Grynkraut: Compt. rend. Soc. de biol. *122*: 835 (1936).
72. S. Wilner & S. Zakrzewski: Presse méd. *1*: 60 (1937).
73. M. J. Eisen & A. Langer: Tumori n.s. *12*: 55 (1938).

been forthcoming which essentially substantiated the possibility of producing tumor immunity by means of intracutaneous inoculations. Thus, out of 180 mice inoculated intracutaneously with Ehrlich sarcoma, eighty-seven succumbed to the tumor, forty-seven developed no tumors at all, and regressions of tumors occurred in forty-six; fify per cent of these refractory animals exhibited a lasting or temporary immunity against subsequent subcutaneous tumor transplantations.[74] Other authors even observed the disappearance of coexisting Brown-Pearce carcinoma growing in the testicles of rabbits, when intracutaneous transplants underwent regression,[75] and the observation that tumor growth was possible in the anterior eye chamber of rabbits previously immunized by intracutaneous inoculation was interpreted as indicating that a barrier exists which prevents the factor responsible for the tumor resistance from reaching the anterior chamber.[76] In addition, in a study of very recent date the findings of Besredka and Gross were confirmed; that is, regression of intracutaneous transplants of Brown-Pearce carcinoma occurred in ninety per cent of the rabbits, and this effect conveyed immunity to them.[77] A definite evaluation of these results, however, cannot be given in view of the divergent reports mentioned, and it must be stressed that the most pronounced effects were obtainable only in one type of tumor, viz., the Brown-Pearce rabbit carcinoma, whereas a general validity of the phenomenon in question for other neoplasms has not been established.

As mentioned before, numerous studies employed unspecific "antigens," i.e., substrates not derived from malignant tumors, for the production of immunity in animals. Very frequently embryonic tissue has been used with apparent success,[78-83] although negative results of such experiments have been reported,[84] and in this connection it must be recalled that this "immunizing" effect has recently been demonstrated as depending on genetic factors.[56] Similar mechanisms may be involved in instances in which sera of foreign species were used,[85] and it seems to be of little relevance whether or not the sera were derived from animals previously treated with tumor tissue.[86] In addition, the injection of homologous or heterologous blood into mice was reported to lower the number of takes and to delay the growth of transplanted tumors.[87] Many studies have been devoted to an antiblastic effect of spleen tissue,[88] a problem discussed in detail in the last part of this chapter; but other organs (muscle, kidney, liver) were also employed with similar success,[89] and in particular, implantations of heterologous organs (spleen, liver, lung, brain, heart-muscle) into mice, prior to inoculation of Ehrlich carcinomas, were observed to protect forty-one per cent of the animals against tumor growth.[90] Moreover, the resistance of animals against tumor transplantations was in-

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[74] A. Bessemans & L. Asaert: Ann. Inst. Pasteur *58*: 130 (1937).

[75] O. Saphir & M. Appel: Am. J. Cancer *38*: 55 (1940).

[76] O. Saphir, M. Appel & A. A. Strauss: Cancer Research *1*: 545 (1941).

[77] F. S. Cheever & C. A. Janeway: Cancer Research *1*: 23 (1941).

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[78] F. Blumenthal: Ztschr. f. Krebsforsch. *27*: 26 (1926).

[79] J. Fibiger & P. Moller: Acta path. et microbiol. Scandinav. *4*: 136 (1927).

[80] V. Carulla Riera: Bol. Cancerol. *1*: 4 (1931); abstr. Ztschr. f. Krebsforsch *37*: Ref. 33 (1932).

[81] V. Carulla Riera & B. A. Cuevas: Arch. espan. oncol. *3*: 155 (1932); 1st Internat. Cancer Congress, Madrid *2*: 371 (1933).

[82] V. Baróni & O. Comsia: Compt. rend. Soc. de biol. *113*: 1310 (1933).

[83] F. C. Pybus: Brit. J. Exper. Path. *15*: 89 (1934).

[84] P. Moller: Acta path. et microbiol. Scandinav. *14*: 228 (1937).

[85] P. Rondoni: Ztschr. f. Krebsforsch. *25*: 455 (1927).

[86] M. v. Eisler & F. Silberstein: Zentralbl. f. Bakteriol. *81*: 269 (1918).

[87] A. A. Kulikowskaja: Arch. f. path. Anat. u. path. Physiol. URSS *4*: 63 (1938); abstr. Am. J. Cancer *40*: 407 (1940).

[88] F. Reichert: Ztschr. f. Krebsforsch. *24*: 255 (1927); *25*: 62 (1927); *27*: 82 (1928).

[89] X. Chahovitch & M. Vichnjitch: Acta Cancrol. *2*: 85 (1936).

[90] G. Tsutsulopulos & E. Lenze: Ztschr. f. Krebsforsch. *49*: 363 (1939).

creased by injections of substances of other origin—for instance, injections of fatty acids,[91] of thorium[92] (cf. chapter I), or of milk.[93] Finally, analogous effects were observed in consequence of a lack of oxygen,[94] and from roentgen irradiation.[95] In connection with the latter statement, reference is made to a publication of more recent date, according to which roentgen irradiation of rat skin impeded a subsequent transplantation of Jensen sarcomas. But it is noteworthy that this inhibition was confined to the primary tumor and did not extend to metastases.[96]

The variety of the agents enumerated, the use of which led to effects closely resembling one another, makes it comprehensible that the idea of a specific nature of these phenomena has been generally abandoned, and that the prevailing opinion interprets them as the expression of an activation of defensive mechanisms in the body which increase the resistance against neoplasia. A common explanation of these effects, which are caused by various agents, has been attempted by Caspari in his theory of necrohormones (cf. chapter VII); that is, by assuming that the disintegration of the injected substances, or the decomposition of body tissues, would result in breakdown products which may stimulate the function of the reticulo-endothelial system.[97] This hypothesis appears to be in agreement with a number of observations and experiments which will be presented later. From a general viewpoint an analogy may be seen between the fact that carcinogenesis, as shown previously, may be induced by a great variety of chemical and physical stimuli (for the action of which so far no common mechanism could be established) and between the phenomena described which demonstrate widely differing stimuli as being capable of counteracting the development and growth of malignant tumors. Moreover, several of the studies enumerated clearly indicated that this increased resistance may be regarded as located mainly in the cellular, and not in the humoral media, a fact to which great significance must be attached in view of the investigations discussed later devoted to the demonstration of tumor-specific immune bodies in blood and other humoral media. Obviously these relations will be of decisive significance for the outcome of experiments, dealt with in the next section, on passive immunization against tumors.

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[91] W. Nakahara: J. Exper. Med. *40*: 363 (1924).

[92] M. Yamauchi: Ztschr. f. Krebsforsch. *21*: 230 (1924).

[93] F. Mandl & F. Stöhr: Wien. klin. Wchnschr. *37*: 1275 (1924).

[94] E. Schwarz: Ztschr. f. Krebsforsch. *21*: 472 (1924).

[95] J. B. Murphy, J. Maisin & E. Sturm: J. Exper. Med. *38*: 645 (1923).

[96] B. Grynkraut & J. Flaks: Bull. Assoc. franç. p. l'étude du cancer *27*: 268 (1938).

[97] W. Caspari & E. Schwarz: Ztschr. f. Krebsforsch. *24*: 15 (1927).

Before concluding the presentation of material on active immunization, reference must be made to some studies which have been undertaken on the basis of a different theoretical approach. Thus vitamin D, complemented by horse albumin as hapten, has been used for an immunization of rabbits against Brown-Pearce carcinomas because of the chemical relationship of the vitamin to carcinogenic phenanthrene derivatives. Approximately thirty per cent of the treated animals were reported to have shown an increased resistance to tumor transplantation,[98] but these results cannot be regarded as being conclusive because of the insufficient experimental material and, in addition, the theoretical premise must be considered with some reserve, since, in spite of the chemical configuration, no evidence is available as to a carcinogenic activity of vitamin D. Much greater significance may be assigned to work in which dibenzanthracene was linked to protein and used as an antigen in the form of 1,2,5,6-dibenzanthracene-carbamido-casein.[99] Treatment of mice with this substance markedly reduced their susceptibility to the carcinogenic action of dibenzanthracene injections when compared with control animals.[100] A continuation of similar experiments may be expected with great interest.

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[98] J. Stefl: Klin. Wchnschr. *16*: 501 (1937).

[99] W. R. Franks & H. J. Creech: Am. J. Cancer *30*: 555 (1937).

Passive immunization. The principle of the studies in this field may be defined, in analogy to general immunological concepts, as the transfer of immune bodies, formed in one animal, into the body of another, for the purpose of protecting the latter against the pathogenic process, i.e., in this case against tumors. Such experiments were started at an early date, and sera of rabbits which had been treated with injections of mouse tumors were found to exert a tumor inhibition when injected into tumor-bearing mice.[101] The injection of nucleoglobulin, isolated from neoplastic tissue, into horses was followed by a high carcinolytic titer of the horse sera, as indicated by vitro experiments, but the therapeutical administration of these sera failed to yield encouraging results.[102] In extensive studies Lumsden [103, 104] endeavored to produce specific immune sera against tumors, and reported that injections of such sera brought about cures in seventy-four per cent of transplanted tumors. It must, however, be remarked that the author employed intratumoral injections of the sera, and that the results obtained with spontaneous animal tumors were far less favorable. Furthermore, objections have been raised against the interpretation of these findings as far as the tumor-specific nature of these immune sera is concerned; these views will be discussed in the next part of this chapter. Generally, treatment of animal tumors by means of passive immunization, i.e., by injections of sera derived from "immunized" animals, only rarely led to satisfactory results.[105, 106] An interesting finding, which to our knowledge has not been re-examined, was announced in which transplantation of Jensen sarcomas to rats in the last stages of pregnancy conveyed a relative immunity against the Jensen sarcoma to the litter of these animals.[107] Of the more recent investigations in this field a negative outcome must be reported, inasmuch as blood derived from rabbits immunized against Brown-Pearce carcinoma did not protect other rabbits against inoculation of this tumor, even when large quantities (70–80 c.c.) of the immune blood were transferred.[108] On the other hand, a more encouraging aspect is exhibited by the results of the following study. The cell nuclei of rat carcinosarcoma 256 were isolated, and emulsions of this material were injected to rabbits weekly for one year; intraperitoneal injections of the serum of these rabbits were administered to rats three weeks after inoculation of the carcinosarcoma 256, and these tumors were found to reach a smaller size, and to be inhibited in their growth, when compared with tumors of control animals. However, in view of the small number of experiments these results were considered by the authors themselves as being only of a preliminary nature.[109] A considerable number of studies in which "specific immune sera" were used for the treatment of human neoplasia will be enumerated in the part of this book dealing with the immunotherapy of malignant tumors, but it may be stressed that the

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[100] W. R. Franks & H. J. Creech: Am J. Cancer *35*: 203 (1939).

[101] C. O. Jensen: Zentralbl. f. Bakteriol. *34*: 28; 122 (1903).

[102] E. Freund: Wien. klin. Wchnschr. *26*: 2108 (1913).

[103] T. Lumsden: Am. J. Cancer *15*: 563 (1931); J. Path. & Bact. *35*: 441 (1932).

[104] T. Lumsden, T. Macrae & E. Skipper: J. Path. & Bact. *39*: 595 (1934).

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105. C. Lewin: Ztschr. f. Krebsforsch. *11*: 335 (1912).

106. Z. Kawakami, F. Nakamura & T. Takei: Jap. Med. World *7*: 127 (1927); abstr. Cancer Rev. *3*: 11 (1928).

107. L. Marx: Ztschr. f. Krebsforsch. *35*: 170 (1932).

108. E. Wollman & M. Bardach: Compt. rend. Soc. de biol. *127*: 858 (1938).

109. C. A. Stoneburg & F. L. Haven: Am. J. Cancer *38*: 377 (1940).

evaluation of both animal and clinical experiments of this kind depends on two salient points. One, it must be decided whether specific tumor-immune bodies are demonstrable as existing in the humoral media; two, a correct interpretation of the effects observed must take into account the fact that they are possibly due merely to heterologous antibodies, formed by the immunized animals in response to treatment with tissue foreign to the species. An elucidation of these problems will be attempted in the following section.

2. *Tumor-Specific Antigens and Antibodies.*

For reasons previously mentioned, the demonstration of tumor-specific immune bodies must be regarded as being of fundamental importance for the whole theoretical aspect of tumor immunology. Furthermore, the pertinent studies have been motivated by pre-eminently practical purposes—on the one hand they seemed to promise an effective cancer therapy, insofar as they might make available "tumor-immune sera" —on the other hand, they were undertaken as a possible means to the end of arriving at a specific tumor diagnosis, which latter aspect will be detailed in chapter X. Actually, it may be anticipated that neither of the two practical aims have been reached, and that even the theoretical clarification of this problem has not yet by any means attained satisfactory results, with the exception of some special types of animal neoplasia, caused by viruses. A frequently chosen approach for the demonstration of tumor antibodies in the sera of tumor-bearing hosts was represented by complement-fixation tests, employing various antigens derived from neoplasms. Negative results of such experiments were reported a long time ago, when aqueous tumor extracts served as antigens,[110] whereas positive findings of complement-fixations were observed in studies using lipid tumor antigens,[111-114] although the percentage of positive results in all these studies must be considered insufficient for a diagnostic application (cf. chapter X). Sera of animals which had received injections of boiled cancer extracts were also reported to contain specific tumor antibodies, evidenced by complement-fixation,[111] and rabbits treated with human gastric carcinoma it was claimed yielded sera which reacted positively not only with the cancer tissue, but also with the gastric contents of patients suffering from cancer of the stomach.[115] Similar positive results were obtained in experiments in which coctostable antigens were derived from the serum or the blood of tumor patients, and were demonstrable by means of complement-fixation tests.[116] The presence of an antigen could be regularly detected in saline extracts of Brown-Pearce rabbit carcinoma; its specific nature was made probable by means of complement-fixation with sera of tumor animals or of animals in which the tumor had receded, but this antigen was found different from those contained in uterine or virus tumors of rabbits.[117] These findings were confirmed by another author [118] although the proportion of the antigen was considerably lower than that reported in the previous investigation. Tumor-specific antibodies were believed to be formed in animals following injections of alcoholic tumor extracts of various neoplasms,[119] but other investigators obtained inconclusive results as to the serologic specificity of antigens in Jensen rat sarcoma,[120] or in Ehrlich mouse carcinoma.[121] In particular, it has been stated that antibodies present in the serum of mice with transplanted tumors cannot be regarded as being specific, since complement-fixation with tumor extracts was not only observed in the sera of these animals but also in the sera of tumor-free mice of higher age.[122] Some studies employed precipitin tests for the demonstration of tumor-immune bodies, a method which was believed to be more suitable for this purpose than complement-fixation reactions.[123] Saline extracts prepared from guinea-pig sarcomas and fractionated by means of Berkefield filtration and adsorption to aluminum hydroxide were injected into hares, and it was reported that the sera, or organ extracts of these animals, contained abundant quantities of precipitins,[124] but their tumor-specific nature does not seem to have been definitely established. Much more significant findings have been derived from a study in which rabbits were injected intramuscularly with human neoplastic tissue; the sera of these animals were adsorbed to "aluminum cream," and this procedure after one to six months removed all precipitins reacting with human blood proteins, whereas the sera retained the property of precipitating specifically and to a high percentage autolysates of human cancer and sixty-seven to eighty-seven per cent of the sera of cancer patients, particularly when the patient was suffering from the same type of tumor as had been used for the production of the immune serum. Furthermore, no precipitins could be detected for animal tumors, when human neoplasms had been used for the immunization, and vice-versa, which facts were interpreted as indicating that the tumor precipitins are species-specific.[125]

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[110] C. S. Engel: Ztschr. f. Krebsforsch. *10*: 248 (1911).

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[111] H. Lehmann-Facius: Ztschr. f. Immunitätsforsch. u. exper. Ther. *67*: 373 (1930).

[112] L. Hirszfeld: Klin. Wchnschr. *8*: 1365 (1929); *9*: 342 (1930).

[113] L. Hirszfeld, W. Halber & J. Laskowski: Ztschr. f. Immunitätsforsch. u. exper. Ther. *61*: 80 (1928).

[114] P. Girard, E. Peyre & H. Boursaus: Bull. Assoc. franç. p. l'étude du cancer *22*: 320 (1933).

[115] C. Cipriani, A. Lucca & A. Allodi: Cancro *1*: 89 (1930); abstr. Ztschr. f. Krebsforsch. *34*: Ref. 21 (1931).

[116] L. H. Peretz & A. I. Newler: Vopr. Onkol. *6*: 246 (1934); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 261 (1935).

[117] J. G. Kidd: Proc. Soc. Exper. Biol. & Med. *38*: 292 (1938).

[118] F. S. Cheever: Proc. Soc. Exper. Biol. & Med. *45*: 517 (1940).

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[119] F. Breinl, V. Kindermann & E. Chrobok: Ztschr. f. Immunitätsforsch. u. exper. Ther. *86*: 260; 274 (1935).

[120] G. D'Alessandro & L. Gaglio: Ztschr. f. Immunitätsforsch. u. exper. Ther. *87*: 137 (1936).

[121] P. Wilke: Ztschr. f. Immunitätsforsch. u. exper. Ther. *87*: 252 (1936).

[122] Sv. Bogdanovic: Ztschr. f. Immunitätsforsch. u. exper. Ther. *98*: 283 (1940).

[123] O. Sievers: Ztschr. f. Krebsforsch. *41*: 307 (1935).

[124] P. Mendeleéff: Compt. rend. Soc. de biol. belg. *136*: 80 (1937).

As a different approach for detecting tumor-immune bodies, experiments with tissue cultures have been utilized, and in this respect it was claimed that the sera of animals immunized against tumors were capable of selectively destroying neoplastic tissues when these sera were added to the cultures.[103, 104, 126] In addition, these immune sera were claimed to exert a certain curative effect when administered to tumor-bearing animals. However, the interpretation of the tissue culture experiments, as far as they were supposed to prove the presence of tumor-specific antibodies in the immune sera, met with numerous objections.[127-130] In particular, the following significant results have been reported. When sheep were injected with defibrinated blood of tumor patients or of healthy persons, or with human tumor substance, and the sera of these animals were employed as media for tissue cultures, a strong growth inhibition was observed. But this effect occurred regardless of whether human tumor tissue, human normal organs, mouse tumors, or normal mouse organs were used. It was stressed that no toxic action was involved, since transfer of the cultures into normal sera was followed by resumption of growth.[131] Other authors also obtained results which disproved the presence of tumor-specific antibodies, as based on observations of tissue-cultures; sera of sheep, immunized against mouse carcinomas, inhibited the growth of tumor tissue and of normal tissue of mice in the same manner;[132] sera of rats, immunized against rat sarcoma, acted similarly in cultures of rat sarcomas or of normal rat spleens,[133] and analogous findings were obtained when rat tumors or normal rat organs were cultivated in sera of guinea pigs immunized against rat tumors.[134] The serum or tissue of "immunized" animals failed to show a cytotoxic action on tissue cultures of Brown-Pearce carcinoma.[134a] Nevertheless, the author of the previously mentioned studies maintained the view that tumor-immune sera also contain, in addition to species-specific and organ-specific antibodies, tumor-specific antibodies, which can be recognized by their action on tissue cultures.[135] In supplementing these vitro experiments, reference may be made to attempts to prove the presence of tumor-immune bodies in vivo, i.e., by means of anaphylactic reactions occurring in tumor hosts when tumor material is administered parenterally. The possibility of inducing such a phenomenon was denied in an older study,[136] but other authors reported on positive findings.[137-142] In

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[125] L. S. Mann & W. H. Welker: Proc. Soc. Exper. Biol. & Med. *43*: 18 (1940).

[126] T. Lumsden: Lancet *1*: 383; *2*: 533 (1935).

[127] F. C. Pybus & H. R. Whitehead: J. Path. & Bact. *32*: 195 (1929).

[128] R. J. Ludford: Proc. Roy. Soc., London s. B *112*: 250 (1930).

[129] P. Mendeleéff: Cancer, Bruxelles *10*: 1 (1933).

[130] L. S. Raposo: Arq. de pat. *5*; 5 (1933); abstr, Am. J. Cancer *22*; 682 (1934).

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131. A. Goldfeder: Acta Cancrol. *2*: 133 (1936).

132. M. J. A. des Ligneris: Pub. South African Inst. Med. Res. *6*: 313 (1934); abstr. Am. J. Cancer *23*: 603 (1935).

133. H. J. Phelps: Am. J. Cancer *31*: 441 (1937).

134. N. Yano: Acta dermat. *33*: 55 (1939).

135. G. O. Favorite & F. S. Cheever: Cancer Research *1*: 136 (1941).

136. T. Lumsden: Am. J. Cancer *31*: 430 (1937); 3rd Internat. Cancer Congress, Atlantic City, 1939.

137. E. Ranzi: Ztschr. f. Immunitätsforsch. u. exper. Ther. *2*: 12 (1909); Wien. klin. Wchnschr. *22*: 372 (1909).

138. H. Pfeiffer & J. Finsterer: Wien. klin. Wchnschr. *22*: 989; 1042 (1909).

139. M. Lapidari: Ztschr. f. Immunitätsforsch. u. exper. Ther. *67*: 159 (1930).

140. P. Chastenet de Géry: Compt. rend. Soc. de biol. *113*: 140 (1933).

particular, the leucocyte drop ("hemoclastic crisis," cf. chapter X) occurring in tumor patients after injection of tumor extracts has been viewed as an allergic reaction, and it was reported that this allergy is passively transferable to healthy individuals.[141] It is obvious that a close connection exists between these allergic reactions and the mechanism of skin tests which have been proposed for tumor-diagnostic purposes, and which will be discussed in chapter X.

Relations of an entirely different nature are apparently involved in those investigations in which a specific infectious agent was held responsible for neoplasia. In this connection, only brief mention is made of a claim that a blastomycetes,[144] or a mucor [145] represents specific "cancer-germs," and that accordingly the sera of cancer patients react in a specific manner with antigens prepared from the respective microorganisms. A scientific value can hardly be assigned to these statements. Yet definite proof has been furnished for the infectious etiology of certain types of neoplasia, viz., the virus tumors of fowl and rabbits, and in these instances the immunological aspects can be expected to differ essentially from those exhibited by other tumor hosts. While an outline of these relations will be given in chapter IX, a few points which appear of interest in conjunction with the preceding remarks need be mentioned here. An "anti-fowl serum," produced in goats, has been found capable of neutralizing the fowl sarcoma agent, that is, injection of such a mixture did not result in tumor formation.[146] Furthermore, an immunological relationship has been established as existing between infectious fowl sarcomas and fowl tumors which had been induced by

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141. Zerner: Ztschr. f. Krebsforsch. *23*: 9 (1925).
142. H. Auler: Ztschr. f. Krebsforsch. *30*: 295 (1930).
143. S. Citelli: Libro de Oro, Buenos Aires, p. 240 (1935).
144. E. Sanfelice: Ztschr. f. Krebsforsch. *7*: 564 (1909).
145. W. Schmidt: Ztschr. f. Krebsforsch. *28*: 545 (1929).
146. R. Knox: Brit. J. Exper. Path. *20*: 301 (1939).

dibenzanthracene, although the latter tumors are not transmissible by tumor filtrates; it was observed that the sera of rabbits, injected with suspensions or filtrates of the dibenzanthracene-fowl tumor, neutralized filtrates of Rous sarcoma #1, thus cancelling its tumor-producing activity. The active factor of this antiserum was found to be not adsorbable to normal fowl tissue,[147] and by means of complement-fixation tests it was demonstrated that "filterable" and "nonfilterable" fowl tumors possessed a common antigen.[148]

Reviewing material presented, one cannot but agree with the conclusions drawn in a monograph on this subject published in 1931, in which it was stated that up to that time no stringent evidence had been furnished to prove the existence of tumor-specific immune bodies,[149] possibly with the exception of the virus tumors. The main difficulty which is encountered in the interpretation of such investigations seems to be represented by the presence in neoplasms of organ-specific and of species-specific antigens, which in the course of immunization experiments lead to the formation of corresponding antibodies, possibly masking the presence of tumor-specific antibodies. Although very interesting results have recently been reported on experimental arrangements which apparently surmount this obstacle,[126] other authors pointed out additional factors which should give rise to a cautious evaluation of the tumor-specificity of immune bodies. Thus, increased antigen quantities were found in Jensen rat sarcoma, and by repeated adsorption to normal tissue, the species-specific antibodies could be removed, while antibodies reacting with the sarcoma were still present. However, a tumor-specific nature of these latter antibodies was not considered as proven, and instead, unspecific changes of the tu-

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147. L. Foulds & L. Dmochowski: Brit. J. Exper. Path. *20*: 458 (1939).
148. L. Dmochowski & R. Knox: Brit. J. Exper. Path. *20*: 466 (1939).
149. H. Sachs; Beihefte z. Med. Klin. *27*: No. 2, p. 82 (1931).

mor lipids were thought to be responsible for the phenomena observed.[150] Furthermore, a serologic resemblance between the antigens contained in Brown-Pearce carcinomas and those of pus was shown, inasmuch as increased concentrations of heterogenetic antigens (Forssman) were detected in both tissues,[151] and, based on comparisons between antigens in carcinomatous and tuberculous tissues, it was assumed that similarities exhibited in these instances may depend on a reactivity common to all necrotizing tissues.[152] Likewise, a study, in which the antigens of rat tumors induced by benzpyrene, were investigated, arrived at the conclusion that the supposedly tumor-specific antigens are common with those of rapidly dying tissues, for it was found that immune sera produced with these antigens reacted with complement-fixation not only with benzpyrene tumors and other rat tumors, but also to a lesser degree with extracts of normal rat organs.[153]

Chemical nature of tumor immune bodies. In spite of the ambiguous state of tumor immunology, as illustrated in the foregoing, several authors attempted to gain an insight into the chemical nature of the supposedly tumor-specific immune bodies. In more recent times, the prevailing opinion has appeared to favor the conception of a lipid nature of tumor antigens, thus considering them as haptens in the sense of Landsteiner. In studies devoted to this problem, alcoholic tumor extracts were fractionated; species-specific and organ-specific antigens were separated from tumor-specific ones, and the latter were demonstrated to be present in the phospholipid fraction.[111, 154] The specific antibodies present in the serum of tumor patients were also assumed to possess a lipid nature, but were stated to be saturated by means of circulating antigens, and therefore only capable of detection when the excess antigens, contained in the globulin fraction, had been precipitated.[155] The results of other workers led to similar conclusions as to a lipid character of tumor antigens,[156, 157, 112, 113] but the findings relating to the antigen content of tumor lipid fractions have been contested.[158] In one study "tumor-specific" antigens were found in the fractions soluble in acetone, whereas the part insoluble in acetone (phospholipids), was declared not to have specific properties.[159] Mention should not be omitted, however, of the fact that the immunological reactivity of lipid tumor extracts has been regarded by some authors as not depending on specific antigens, but has rather been viewed as an expression of unspecific chemical and physico-chemical alterations,[160] views which will be discussed in chapter X in connection with "immunological" and "lability" tests for tumor diagnosis. In fact, unspecific changes of the lipid structure have been believed to be responsible for the antigenic quality of Jensen sarcomas,[150] and in continued work the alterations were defined as increases of lecithin and other phospholipids.[161]

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[150] G. D'Alessandro & E. Saladino: Ztschr. f. Immunitätsforsch. u. exper. Ther. *93*: 27 (1938).

[151] L. Dmochowski: Ztschr. f. Immunitätsforsch. u. exper. Ther. *93*: 311 (1938).

[152] L. Dmochowski & A. Prusczcyński: Ztschr. f. Immunitätsforsch. u. exper. Ther. *93*: 331 (1938).

[153] L. Dmochowski: Am. J. Cancer *37*: 252 (1939).

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[154] H. Lehmann-Facius: Klin. Wchnschr. *9*: 2430 (1930); Ztschr. f. Immunitätsforsch. u. exper. Ther. *75*: 217 (1932); *82*: 99 (1934); Frankfurt. Ztschr. f. Path. *47*: 285 (1934).

[155] H. Lehmann-Facius: Ztschr. f. Immunitätsforsch. u. exper. Ther. *51*: 196 (1927); Klin. Wchnschr. *11*: 775 (1932).

[156] E. Witebsky: Klin. Wchnschr. *9*: 58 (1930).

[157] E. Witebsky & P. Pöplau: Ztschr. f. Immunitätsforsch. u. exper. Ther. *76*: 82 (1932).

[158] E. Morelli: Ztschr. f. Immunitätsforsch. u. exper. Ther. *83*: 521 (1934).

[159] A. Zacho: Ztschr. f. Krebsforsch. *44*: 79 (1936).

[160] F. Klopstock & E. Simon: Ztschr. f. Krebsforsch. *37*: 127 (1932).

[161] G. D'Alessandro & A. Greco: Ztschr. f. Immunitätsforsch. u. exper. Ther. *94*: 147 (1938).

On the other hand, some evidence has been presented which might be compatible with older assumptions, which ascribed a protein nature to tumor immune bodies. Such an interpretation may be applied to phenomena discovered in the course of work with the Fuchs test (fibrinolytic reaction, cf. chapter X); that is, it was observed that the sera of tumor patients not only failed to split certain substrates of protein nature, but that under these conditions a decrease of incoagulable nitrogen even takes place in the serum employed. This phenomenon may be due to a precipitation in the serum of nitrogenous substances and, accordingly, indicates the involvement of an antigen-antibody system of protein-like nature.[162] A protein nature has also been made probable for antigens derived from Brown-Pearce rabbit carcinomas,[163] and precipitins for proteins have been produced by immunizing rabbits with human neoplasms.[125] Furthermore, it was reported that the antibodies in tumor-immune sera, the action of which on neoplastic tissue cultures was studied, are present in the euglobulin fraction, from which they may be concentrated in a stable form.[164] However, it must be borne in mind that these results do not necessarily prove the protein character of the active factors, since substances of other chemical constitution may also be adsorbed to protein fractions.

A novel conception was the one which, based on animal experiments, considered the antigenic principle of neoplasms as a complex carbohydrate;[165] later work, however, does not seem to have supported the validity of this view.[166]

Complement content. This problem belongs to a group of studies in which, in addition to the specific tumor immune bodies, aspects of a general immunological nature have been investigated. In this connection, several workers reported they had found an increase of complement in the serum of tumor hosts. Such findings were regularly obtained in the sera of tumor-bearing rats, while sera of tumor patients showed a complement augmentation only in approximately sixty per cent.[167, 168] It is of interest that the authors of these communications were inclined to make this complement increase responsible for the behavior of tumor sera in the so-called lability tests (cf. chapter X). The requirement of active complement for the carcinolytic reaction, demonstrated in some studies, will likewise be discussed in the chapter on tumor diagnostics. A considerable increase of complement has been found in pleura exudates of patients suffering from mammary cancer,[169] and, in contrast to normal complement, the complement of carcinomatous exudates was found to be very stable. It was supposed that the latter originated from autolysed tumor cells and is stabilized by increased fibrinogen concentrations.[170] However, the general occurrence of elevated complement concentrations in neoplasia does not appear proven, since no changes of the complement content were detected in guinea pigs bearing transplanted tumors.[171]

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[162] H. J. Fuchs & H. Kowarzyk: Ztschr. f. Immunitätsforsch. u. exper. Ther. *80*: 375 (1933); Klin. Wchnschr. *12*: 1288 (1933).

[163] J. G. Kidd: J. Exper. Med. *71*: 351 (1940).

[164] T. Lumsden: Biochem. J. *28*: 1368 (1934).

[165] F. Silberstein: Schweiz. med. Wchnschr. *62*: 153 (1932).

[166] W. R. Franks: Tr. Roy. Soc. Canad. Biol. Sc., s. III *27*: 135 (1933).

Heterogenetic antigens. These factors, named after Forssman, represent antigens which lead to the production of hemolysins which act upon red blood cells of foreign species. The presence of such antigens in tumors was demonstrated a long time ago in mouse carcinomas,[172, 173] and heterogenetic

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167. K. Pelczar & F. Hofbauer: abstr. Ztschr. f. Krebsforsch. *38*: Ref. 85 (1932).
168. K. Pelczar: 1st Internat. Cancer Congress, Madrid *1*: 209 (1933).
169. H. Auler & P. Meyer: Ztschr. f. Krebsforsch. *30*: 286 (1929).
170. H. Auler, H. Schlottmann, W. Rubenow, P. Meyer & B. Wolff: Ztschr. f. Krebsforsch. *32*: 186 (1930).
171. J. Gordon & J. W. Orr: J. Path. & Bact. *46*: 209 (1938).
172. J. Morgenroth: Berlin. klin. Wchnschr. *50*: 561 (1913).
173. J. Morgenroth & R. Bieling: Biochem. Ztschr. *68*: 85 (1915).

antigens were later detected in Rous sarcomas[174] and also in the blood of cancer patients,[175] which latter finding stimulated diagnostic experiments. A probable connection exists between this observation and an older claim, according to which the sera of tumor hosts contain hemolysins and precipitins for red blood cells and proteins derived from foreign species.[176] The theoretical premise, however, of the author, who assumed the existence of a serologic identity of tumor protein and proteins of foreign species, has been rejected and the diagnostic usefulness of the proposed test has been contested, as mentioned in chapter X. More recently, as remarked above, increased amounts of heterogenetic antigens were described as present in Brown-Pearce carcinomas, and also in pus and tuberculous tissues, and hence these findings were considered as bearing no specific relation to tumor immunology, but were considered to be a feature common to rapidly dying tissues.[151, 152]

Hemagglutinins. The question has repeatedly been considered as to whether deviations from the normal could be detected concerning the distribution of isoagglutinins in cancer patients. However, determinations of the blood groups failed to reveal characteristic differences, as shown in a study of 300 tumor patients; this finding concurs with the results of previous investigations.[177] Interesting observations have been communicated regarding the agglutination of rat erythrocytes. Normally, the serum of rats does not contain isoagglutinins, but these factors can be produced by injections of heterologous or autologous tissue. On the other hand, the red blood cells of rats contain agglutinogens, and an inverse ratio was found to exist in animals between the ability to produce agglutinins and the tendency of their erythrocytes to agglutinate; that is, rats with red blood cells which are easily agglutinated, produce less agglutinin than do those animals in which the agglutination of erythrocytes occurs less readily. A connection of these qualities to tumor pathology has been established, inasmuch as rats with high susceptibility to Jensen sarcoma were found regularly to possess red blood cells which could be easily agglutinated, and the opposite behavior was found in tumor-resistant animals. Furthermore, the sera of rats bearing rapidly growing sarcomas were never found to contain agglutinins, while these factors were always present in the serum of resistant animals, or of those in which tumors had regressed. Hence it was deduced that the natural resistance of rats against Jensen sarcomas runs parallel to their ability to form isoagglutinins, and this reactivity was believed to be usable for a selection of animals for experimental purposes.[178]

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[174] P. Gerėb & R. Simke: Ztschr. f. Krebsforsch. *35*: 73 (1931).

[175] L. de Kromme & J. R. de Bruine Groeneveldt: Proc. Roy. Acad. Amsterdam *36*: 463 (1933); abstr. Ztschr. f. Krebsforsch. *40*: Ref. 54 (1933).

[176] G. Kelling: Berlin. klin. Wchnschr. *42*: 911; 950 (1905); Ztschr. f. Krebsforsch. *4*: 439 (1906).

[177] A. Goldfeder & J. L. Fershing: Am. J. Cancer *29*: 307 (1937).

The claim has been advanced that a characteristic difference relating to hemagglutinins exists in tumor patients, inasmuch as auto-agglutinins were believed to be regularly present in the sera of these patients.[179] However, a re-examination of this problem failed to corroborate the original findings.[180]

"*Tumor toxins.*" A purely speculative approach has frequently been made, by supposing that specific immune bodies might be involved in the clinical manifestation of cachexia which characterizes the appearance of tumor patients, particularly in later stages of the disease. However, neither older work[181] nor evidence of recent times has proved such an assumption, i.e., that neoplasms produce specific "toxins" responsible for tumor cachexia. In this connection, most probably no primary significance can be attached to a report on the presence of toxic substances in the urine of rats with Jensen sarcomas,[182] nor to the findings regarding abortifacient agents in the urines of tumor hosts, which phenomena will be detailed in Chapter X. Most probably the active factors in these instances are represented by products of protein breakdown, i.e., they constitute a secondary consequence of the tumor process. As to the causation of cachexia in neoplasia, it appears plausible to hold a number of factors responsible for this phenomenon. Nutritional disturbances, the effect of large-scale tissue disintegration, involvement of various organ functions in general, and of endocrines in particular, apparently contribute to the composite picture manifested clinically.

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[178] T. Lumsden: Am. J. Cancer *32*: 395 (1938).

[179] P. Neuda: Ztschr. f. Immunitätsforsch. u. exper. Ther. *86*: 393 (1935); *89*: 76 (1936); *90*: 493 (1937).

[180] E. Poulsen: Ztschr. f. Immunitätsforsch. u. exper. Ther. *90*: 134 (1937); *92*: 98 (1938).

3. *Specific Immunotherapy of Neoplasms.*

No misapprehension should arise as to the writers' opinion regarding the material to be presented in the following; that is, it is not implied that a specific immunotherapy of malignant tumors has either been accomplished or can definitely be expected to find realization in the future. Yet, in spite of the failures or unsatisfactory results of theoretical tumor immunology, described in the preceding parts, attempts to utilize experiences and procedures drawn from general immunology for treatment of malignant tumors must be recorded. But at the same time it should be borne in mind that even in some instances of apparent success, no conclusive proof is available inferring a specific nature—in the sense of classic immunology—of the procedures involved. Hence the division made here between "specific" and "unspecific" immunotherapy refers less to their intrinsic nature than to the theoretical foundation or to the interpretation applied to them by their authors.

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[181] C. Lewin: Ergebn. d. inn. Med. u. Kinderh. *2*: 168 (1908).

[182] B. Purjesz, L. Berkessy & K. Gönczi: Ztschr. f. Krebsforsch. *41*: 358 (1935).

As remarked in the first part of this chapter, animal experiments show that an active immunization against tumors is in general feasible only by employing virulent neoplastic tissue, and it is comprehensible that such a procedure could not readily be initiated for clinical purposes. However, as an attempt in this direction, the undertaking, consisting of implanting intracutaneously tumor suspensions obtained by partial removal of inoperable uterine carcinoma, may be considered; this procedure was reported to cause a growth inhibition of the primary uterine tumor.[183, 184] Similarly, favorable effects were believed to result from the injection of autologous or homologous tumor emulsions into patients with cancer of the female genital tract, either following surgical removal of the tumor, or in combination with radiation therapy.[185] An interesting experiment was performed by employing roentgen-irradiated tumor suspensions for the treatment of inoperable cancer patients,[186] but no conclusive results were obtained and, to our knowledge, further experiences with this method have not been recorded. A novel proposal for cancer prophylaxis, which may be regarded as related to active immunization, has been advanced in the form of producing, artificially, skin cancers, which could be cured comparatively easily, and which would protect the patient against the occurrence of internal malignant tumors.[187]

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183. J. Thies: München. med. Wchnschr. *71*: 1054 (1924); *72*: 2082 (1925); Arch. f. Gynäk. *125*: 517 (1925).

184. E. Knopf: Ztschr. f. Krebsforsch. *32*: 305 (1931).

185. S. Ohno & K. Morooka: Tr. Jap. Path. Soc. *16*: 262 (1928); *17*: 549 (1929); abstr. Ztschr. f. Krebsforsch. *31*: Ref. 13 (1930).

186. T. H. Kellock, H. Chambers & S. Russ: Lancet *1*; 217 (1922).

187. S. Peller: Lancet *2*: 552 (1936).

The rationale underlying this idea is derived from statistics which show an inverse ratio as existing between the incidence of skin cancer and cancer of internal organs, as will be discussed in detail in chapter IX, part I.

On a much broader scale, although with scarcely more encouraging results, experiments have been undertaken employing extracts or decomposition products of neoplasms instead of native tumor substances; tumor autolysates have most frequently been used. This trend was started by Keysser[188] and apparently was influenced by concepts of vaccination employed for prophylaxis in certain infectious diseases. Several authors elaborated the therapy by means of tumor autolysates, and believed that in this manner antiblastic forces may be mobilized in the body, which prove useful for prophylaxis or the treatment of neoplasia.[189-191] Numerous reports, some of them favorable, have been published on this subject, but the successes concerned only certain individual cases without permitting the assumption of a general effectiveness of this therapy.[192-202] Most frequently subcutaneous and intramuscular injections of the preparations have been used, but intravenous administration has also been tried and has been reported to yield some beneficial results,[196, 198] although the dangers inherent in this procedure were stressed.[198] The question as to the mechanism involved in those instances where this treatment brought about undeniable improvements has been answered in different ways. The presence of choline in autolysates was held responsible ([193,] cf. chapter II), while a proteolytic activity of the substrates was believed by another author to represent the curative factor.[198] A growth inhibition of tissue cultures was observed, following the addition of autolysates of normal organs, and neoplastic cells were found to be more sensitive than normal ones.[201] These latter findings strongly suggest that the effects observed depend principally on more or less unspecific growth inhibitions, due to the action of protein cleavage products. This view seems supported by the results of animal experimentation, in which autolysates or tumor extracts exerted either an inhibiting or a stimulating influence on transplanted tumors, in accordance with different experimental arrangements,[199, 203] a phenomenon which may explain the obvious failure of some clinical investigations.[192–195] Other tumor substrates have been used with similar results; thus good results were reported from the administration of tumor extracts in tumor-bearing animals [204] or in human malignancy.[194] Cancerous tissue was disintegrated by means of high-pressure steam and the resulting product—called "atmide albumin"—was reported to cause clinical improvements in some tumor patients,[205] and similar favorable results were claimed as being achieved by the parenteral administra-

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[188] F. Keysser: Munchen. med. Wchnschr. *61*. 33 (1914); Vakzinebehandlung der bösartigen Geschwulste, Hirzel, Leipzig 1923.

[189] F. Blumenthal: Ergebnisse der experimentellen Krebsforschung, Leiden 1934.

[190] C. Lewin: Therapie der Gegenwart *54*: 253 (1913); Ztschr. f. Krebsforsch. *11*: 317 (1912); *32*: 128 (1930).

[191] G. Fichera: Ztschr. f. Krebsforsch. *36*: 1 (1932).

[192] A. Pinkuss & Kloninger: Berlin. klin. Wchnschr. *50*: 1941 (1913).

[193] J. Sellei: Ztschr. f. Chemother. *1*: (1913); abstr. Ztschr. f. Krebsforsch. *13*: 398 (1913).

[194] Lunckenbein: München. med. Wchnschr. *41*: 1047 (1914).

[195] E. Pflaumer: Berlin. klin. Wchnschr. *51*: 867 (1914).

[196] G. Schubert: Berlin. klin. Wchnschr. *51*: 1876 (1914).

[197] R. Bauer, R. Latzel & E. Wessely: Ztschr. f. klin. Med. *81*: 420 (1915).

[198] E. Bosch: Ztschr. f. Krebsforsch. *16*: 325 (1919).

[199] H. Chambers & G. M. Scott: Brit. J. Exper. Path. *5*: 1 (1924).

[200] J. Lamprecht: Dermat. Ztschr. *40*: 313 (1923).

[201] A. H. Roffo & J. B. Villanueva: Bol. inst. méd. exper. para el estud. y trat. d. cáncer *2*: 347 (1926).

[202] J. Thomas: Compt. rend. Acad. d. sc. *184*: 1592 (1927).

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203. G. Domagk & Ch. Hackmann: Ztschr. f. Krebsforsch. *42*: 192 (1935); Zentralbl. f. Path. *63*: Erg.-H., 116 (1935).
204. E. Berger: Ztschr. f. Krebsforsch. *20*: 10 (1923).
205. R. Bauer & W. Nyiri: Wien. klin. Wchnschr. *34*: 520 (1921).

tion of enzymatic split products of tumor tissue.[206-208] Special methods of enzymatic tumor fission have been devised, leading to a preparation called "ultrapeptone," [209] the use of which was reported by one author to yield remarkable results in human neoplasia.[210] Phosphotungsten precipitates of tumor hydrolysates have been successfully used for cures of mouse tumors, particularly when homologous substrates were employed,[211] but no clinical application of this procedure has been recorded. In accord with the assumption of a lipid nature of the supposed tumor antigens, therapeutic use has also been made of tumor lipids or of lipid-protein mixtures,[212-214] but the effects observed do not seem to indicate a superior value of these measures.

In direct analogy to the immunotherapy of infectious diseases, a passive immunization of tumor patients has most frequently been attempted by means of producing and administering an "anti-cancer-serum." These efforts go back more than thirty years, and for an extensive bibliography, reference is made to reviews of older [215] or more recent date.[86, 216, 217] A distinction must be made between two groups of "antisera," i.e., according to whether they are supposed to be directed against the tumor cell, or against certain alleged "cancer-germs." The results reported with preparations of

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206. G. Joannovics: Wien. klin. Wchnschr. *33*: 649 (1920).
207. G. Scherber: Wien. klin. Wchnschr. *33*: 654 (1920).
208. X. Chahovitch: 1st Internat. Cancer Congress, Madrid *1*: 485 (1933).
209. U. Rebaudi: Riforma med. *46*: 949 (1930).
210. P. Ettienne-Martin: 1st Internat. Cancer Congress, Madrid *1*: 447 (1933).
211. R. Werner & H. Winter: Ztschr. f. Krebsforsch. *39*: 89 (1933).
212. P. Rondoni: Ztschr. f. Krebsforsch. *27*: 497 (1926).
213. H. Auler & K. Pelczar: Ztschr. f. Krebsforsch. *27*: 104 (1926).
214. H. Auler: Ztschr. f. Krebsforsch. *30*: 295 (1928).
215. C. Lewin: Ergebn. d. inn. Med. u. Kinderh. *8*: 499 (1912).
216. W. Nyka & J. Lavedan: Paris méd. *1*: 229 (1934).
217. C. Richet: Libro de Oro, Buenos Aires, p. 1192 (1935).

the latter kind—e.g., with "blastolysin," a serum directed against a myxomycetes[218]—must be evaluated with great skepticism in view of the completely lacking scientific approach, and because of the fact that there is ample reason to believe that many of the allegedly "cured" cancer patients in reality were, to begin with, free of malignancy. But, likewise, with the other group of anti-cancer-sera, therapeutical effects of only moderate or ambiguous nature have been accomplished. On the other hand, the fact that temporary clinical improvements occasionally resulted from such treatments cannot be surprising, since any introduction of heterologous protein results in a stimulation of natural defensive mechanisms and consequently may cause a temporary growth inhibition of neoplasms. In supporting this view, mention is made of the circumstance that the effect of such sera was much more pronounced in animals with transplanted tumors—that is, in an organism with intact reactivity—rather than in human tumor patients, the resistance of whom had been severely impaired by the disease. Nevertheless, endeavors to treat human neoplasia with anti-sera were continued until recent times; the fractionation of such sera and the concentration of the active factors have been performed,[164] or the use of pregnant animals for the production of immune sera has been recommended,[219] a proposal the rationality of which is somewhat obscure. On the basis of endocrine aspects, sera have been tried which were directed not against the tumor, but against the sex glands, and favorable effects of their administration were claimed in the treatment of skin cancer.[220] More recently serum therapy in human cancer has been resumed on a broader scale. Two procedures were tried: one group of patients received intramuscular injections of thirty to forty c.c. of serum of healthy persons, and some general improvement and gains in weight were observed, with sometimes also a slight diminution of the tumor size. Another group of cancer cases was treated with injections of the sera of sheep, which had been immunized with defibrinated blood of carcinomatous and sarcomatous patients. In these instances beneficial effects of a general nature also resulted, and the serum therapy appeared to improve the results of simultaneous radiation therapy. A concentration of the active factors contained in the sera was considered to be a possible means for increasing the effectiveness of the therapy.[221]

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[218] J. Pick: Prakt. Karzinombl. *3*: No. 2 (1935); Ars medici *14*: Jan. 1936; Med. Times, Oct.-Nov. 1936.

[219] V. Ghiron: Sperimentale *83*: 121 (1929); abstr. Cancer Rev. *4*: 600 (1929).

[220] Deutschmann: Klin. Wchnschr. *1*: 398 (1922).

4. *Unspecific Immunotherapy.*

As stated before, therapeutic attempts to be discussed in this part have been, a priori, undertaken with a knowledge of their unspecific nature, but it is reiterated here that most probably many measures previously enumerated, and originally regarded as being specific, actually owe their effect to an unspecific activation of the body resistance against the tumor process.

One of the simplest stimulation therapies employed in various pathologies is the use of autologous blood (auto-transfusion), i.e., the intramuscular re-injection of blood which has been withdrawn from the patient himself. Since this method was proposed by Bier, it has frequently been clinically applied in neoplasia.[222] As was to be expected, its success consisted of transient improvements, observed only in a certain percentage of the cases treated. A peculiar combination of auto-transfusion with radiation therapy is represented by the re-injection of blood which previously has been exposed to roentgen or radium radiation.[222-224] The underlying idea may have been that radiation therapy proper affects the tumor not only locally but also brings about general effects by means of an alteration of the humoral media. One of the studies mentioned, however, was based on the assumption that under the action of radium a "cancer-cell-protecting factor," present in the serum of cancer patients, is transformed into a carcinolytic factor ([223], cf. chapter X). No definite evaluation of this treatment and of similar procedures —injections of ascites fluid of cancer patients[225]—appears possible, since favorable opinions[224] are contrasted by negative ones.[226]

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[221] I. I. Kaplan & A. Goldfeder: Am. J. Roentgenol. *39*: 84 (1938).
[222] G. Fichera: loc. cit. 6, p. 45.

One of the most important biological stimuli, viz., bacterial infection, has acquired significance in connection with cancer curative measures, starting with the clinical observation that an incidental erysipelas influenced the course of neoplasia, in some instances, in an amazingly favorable manner.[227] Accordingly, erysipelas infections have been induced artificially in tumor patients, and this procedure seemed actually to impede neoplastic growth and to prolong the life of the patient.[228-230] However, a more recent study urged a cautious evaluation of this therapy, because misleading impressions might be gained from the literature, in which only successfully treated cases are described, whereas the failures which resulted have most frequently been withheld from publication.[231] A more easily reproducible method, involving fewer dangers than the erysipelas infection, has been devised by Coley,[232] in the form of injections of a toxin mixture prepared from Bacillus erysipelatos and Bacillus prodigiosus (Coley's fluid). Remarkable results of this treatment were seen especially in bone sarcomas and several workers, in the essentials, confirmed the beneficial effects of this therapy.[233-235] However, animal experimentation showed that intraperitoneal injection of Coley's fluid to rats bearing sarcomas induced by benzpyrene or methylcholanthrene failed to bring about a marked inhibition of tumor growth, although hyperemic and hemorrhagic reactions occurred in the sarcomas.[236] The latter phenomenon, i.e., hemorrhages occurring in tumors, following bacterial infection, has been thoroughly analyzed and is named, after the author of these studies, the "Shwartzman phenomenon." [237] This author demonstrated that these effects could be produced by the infection of tumor-bearing animals with bacteria of the coli group, particularly Bacillus enteridis, and he also demonstrated that injections of bacterial filtrates exerted a similar action, resulting in some instances in the inhibition or even the regression of animal tumors. Numerous authors confirmed these findings by using bacterial filtrates or extracts (Bacillus proteus, Bacillus typhosus, Bacterium coli) [238-242] but this effect was found to be limited to transplanted animal tumors (mouse sarcoma 180).[240] No definite evaluation can as yet be given to the assumption that a sudden drop of the Vitamin-C content in the tumors is responsible for the Shwartzman phenomenon, or is connected with it.[243] Other workers, who produced the Shwartzman phenomenon in mouse sarcoma 180 by means of injections of filtrates of Salmonella typhi murium, observed that this treatment caused cures in a high percentage of cases, and they demonstrated polysaccharides and nucleoproteins as being the active factors responsible for the effects.[244] Yet, in another investigation in which the Shwartzman phenomenon was elicited in transplanted mouse and rat tumors, by means of filtrates of coli and meningococcus, the hemorrhages resulting from this treatment were seen to cause only a temporary arresting of growth, but no regression of the tumors was caused.[245] Mice bearing transplanted carcinomas received roentgen irradiation, and were simultaneously treated with intraperitoneal injections of Coli and Paratyphoid B toxins. This combined therapy, however, was not found to yield better results, as to regression of tumors and as to prolongation of life, than roentgen irradiation alone.[245a] Accordingly, it may be assumed that the action of these bacterial products has so far not been shown to influence neoplasms in a manner which would warrant a clinical application of this therapy, which has been tried only occasionally.[238] A similar attitude may be maintained regarding several other bacterial substances, which have been investigated as to their curative effect on malignant tumors. Tuberculin injections have been reported to bring about a diminution of the tumor size and prolongation of life in seven inoperable

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[223] H. Peters: Ztschr. f. Krebsforsch. *28*: 186 (1928).

[224] S. v. Dziembowski: Fortschr. d. Med. *48*: 567 (1930).

[225] C. Lewin: Berlin. klin. Wchnschr. *56*: 1233 (1919); Ztschr. f. Krebsforsch. *32*: 128 (1930).

[226] J. Allmann: Strahlentherapie *4*: 626 (1914).

[227] Fehleisen: Deutsche med. Wchnschr. *8*: 553 (1882).

[228] O. Lassar: Deutsche med. Wchnschr. *17*: 898 (1891).

[229] C. H. H. Spronck: Ann. Inst. Pasteur *6*: 683 (1892).

[230] Wolffheim: Berlin. klin. Wchnschr. *58*: 1012 (1921).

[231] C. Rosenrauch: Clin., Paris *28*: 324 (1933); abstr. Am. J. Cancer *22*: 141 (1934).

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[232] W. B. Coley: Ann. Surg. *14*: 199 (1891); Cancer Rev. *4*: 425 (1929); Ann. Surg. *97*: 438 (1933); Glasgow M. J. *126*: 49 (1936).

[233] S. P. Beebe & M. Tracy: J. A. M. A. *49*: 1493 (1907).

[234] P. Uhlenhuth, M. Haendel & K. Steffenhagen: Ztschr. f. Immunitätsforsch. u. exper. Ther. *6*: 654 (1910).

[235] M. Beck: Ztschr. f. Krebsforsch. *10*: 149 (1911).

[236] A. Brunschwig: Ann. Surg. *109*: 109 (1939).

[237] G. Shwartzman: Proc. Soc. Exper. Biol. & Med. *32*: 1603 (1935); Arch. Path. *21*: 284; 509 (1936).

[238] L. H. Peretz, A. I. Newler & L. T. Larionow: Ztschr. f. Krebsforsch. *42*: 417 (1935).

[239] M. Jacobi: Am. J. Cancer *26*: 770 (1936).

[240] H. B. Andervont: Am. J. Cancer *27*: 77 (1936).

[241] L. C. Fogg: Pub. Health Rep. *51*: 56 (1936).

[242] M. J. Eisen: Am. J. Cancer *32*: 30 (1938).

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243. H. B. Andervont & M. B. Shimkin: Am. J. Cancer *36*: 451 (1939).
244. E. L. Walker & M. S. Handman: J. Immunol. *37*: 507 (1939).
245. R. Tulasne: Bull. Assoc. franç. p. l'étude du cancer *28*: 527 (1939).
245a. E. A. Lawrence & F. Duran-Reynals: Yale J. Biol. & Med. *14*: 177 (1941).

cancer patients,[246] while another author only observed subjective improvements of the patients from this treatment.[247] Inhibitions of the growth of various transplanted rat and rabbit tumors have been claimed to result from tuberculin injections.[248] For prophylactic purposes, administration of typhoral, viz., an attenuated toxin of Bacillus typhosus, has been recommended [249] but the meager experiences collected do not seem to support the usefulness of this measure. Furthermore, an antagonism between malaria infection and neoplasia has been supposed to exist because of an allegedly rare incidence of malignant tumors in countries with endemic malaria. In addition to the doubtful value of these statistics, clinical experiments with malaria infection in tumor patients led to only slightly encouraging results.[250] A more promising aspect has been believed to be exhibited by the therapeutic use of another pathogenic microorganism, namely, Schizotrypanum (Trypanosoma) cruzi; animal tumors were reported to be cured, following an infection with this microorganism, or following injection of its endotoxins. This effect was substantiated by histological examination, and was supposed to be specific for the Trypanosoma cruzi, since neither Trypanosoma equiperdum, Spirochaeta Duttoni, nor bacterial toxins of other origin (Pyrifer) showed a similar activity. The antagonism between the infection in question and the tumor process was also expressed by the fact that infected tumor animals had a longer duration of life than noninfected tumor animals and than infected tumor-free animals. Since a prophylactic endotoxin treatment impeded the development of transplanted tumors, it was assumed that the phenomena observed depended on a stimulation of defensive mechanisms in the body (reticulo-endothelial system). Some clinical experiments with this treatment in inoperable tumor patients have been described as yielding beneficial results, but a final evaluation of these statements does not as yet appear possible.[251, 252] Far reaching claims have been advanced as to the therapeutical effectiveness of filtrates of Bacillus histolyticus cultures (ensol), the active factors of which were believed to be proteolytic enzymes.[253] However, as stated in chapter IV, this therapy has met with justified objections and, moreover, experiments with Brown-Pearce rabbit carcinoma failed to show any curative value of ensol.[254]

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[246] R. Pearl, A. C. Sutton & W. T. Howard: Lancet *1*: 1078 (1929).

[247] I. Holmgren: Schweiz. med. Wchnschr. *65*: 1203 (1935); Acta med. Scandinav. *87*: 521 (1936).

[248] S. Imamura: Jap. J. Obst. & Gynec. *20*: 438; 444; 445 (1937); *21*: 107; 111; 115; 123 (1938).

[249] C. Jacobsen: Arch. f. Dermat. *169*: 562 (1934).

[250] A. Braunstein: Ztschr. f. Krebsforsch. *29*: 330; 486 (1929).

The evidence presented in the foregoing indicates that infectious diseases or products of bacterial metabolism may exert a certain inhibitory influence on the neoplastic process, and this view has been supported by theoretical considerations of a somewhat different nature. Even a long time ago statistical analyses suggested the existence of a connection between infectious diseases, contracted by patients in the past, and between the incidence of malignant tumors in their later life; that is, individuals who, in their early life, had been spared infections were believed to later show a higher frequency of neoplasia than persons who had been subject to a larger number of infectious diseases.[255] Although the validity of the statistical material has been doubted,[256] similar opinions and conclusions concerning the relation between

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251. G. Roskin & K. Romanowa: Acta Cancrol. *1*: 323 (1935); Ztschr. f. Krebsforsch. *44*: 375 (1936); Arch. Internat. d. méd. exper. *13*: 379 (1938).
252. G. Roskin, K. Romanowa & A. Maslowa: Vestn. Rentgenol. *16*: 18 (1936); abstr. Ztschr. f. Krebsforsch. *45*: Ref. 336 (1937).
253. H. C. Connell: Canad. M. A. J. *33*: 364 (1935).
254. W. T. Pommerenke: J. A. M. A. *106*: 1654 (1936).
255. R. Schmidt: Med. Klin. *6*: 1690 (1910).
256. S. Peller: Wien. klin. Wchnschr. *48*: 111 (1935).

infections and tumor incidence have been expressed more recently by several authors.[249, 257, 258] The interpretation applied to these observations was based on the assumption that, in persons who have remained comparatively free of infections, the defensive apparatus of the body did not undergo a sufficient development, and hence may fail to protect the organism in the critical moment of initial tumor development. In accordance with present-day knowledge, and in view of numerous facts amplified in the last part of this chapter, it appears justifiable to substitute for the general nomenclature of defensive mechanism the more clearly defined term "functional activity of the reticulo-endothelial system." For it has been established that this cell apparatus is affected by infections and probably also by other obnoxious stimuli, and is responsible for the protection of the body against them. Hence it may be hypothetically presumed that the tumor-inhibiting effects of pathogenic microorganisms and their products enumerated are brought about in an indirect manner, by increasing the natural resistance of the body, and such an assumption makes it comprehensible that the effects observed may vary according to the reactivity of the organism and the virulence of the infection. Such a relationship has actually been proven concerning tuberculous infection—both in animal experiments with Rous sarcoma[259] and on the basis of clinical analyses.[260] Moreover, the local action of microorganisms must be distinguished from their general effect on the body, inasmuch as it has been declared that the bacterial infection of pathologically changed tissue facilitates tumor development.[261] On the other hand, it is commonly known that even closed tumors are very frequently found in-

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257. P. Engel: Wien. klin. Wchnschr. *47*: 1118 (1934); *48*: 112 (1935).
258. F. Sinek: Ztschr. f. Krebsforsch. *44*: 492 (1936).
259. O. Teutschlaender: Ztschr. f. Krebsforsch. *30*: 498 (1928).
260. G. Heddaeus: Ztschr. f. Krebsforsch. *42*: 140 (1935).
261. J. Ewing: Libro de Oro, Buenos Aires, p. 375 (1935).

fected, a fact which has been explained by assuming that the decreased oxygen consumption of neoplastic tissue creates favorable conditions for the growth of certain pathogenic microorganisms.[262] Possibly a connection also exists between the susceptibility of tumors to infection, and the finding that the bactericidal power of serum, as tested with Bacterium coli and Bacillus typhosus, is markedly diminished in tumor-bearing animals.[263] This property may in turn be interpreted to be the expression of a lowered general resistance in neoplasia.

In addition to bacterial toxins, poisonous products of higher animals have recently attracted the attention of oncologists, particularly since the general pharmacology of these substances has made greater progress. The main examples of this group are snake venoms, in particular cobra venom. Setting aside older, inconclusive experiments,[264] it was more recently announced that administration of cobra venom to cancer patients resulted in a striking alleviation of pains;[265-267] some authors reported to have observed in animal experiments growth inhibitions and even regressions of tumors following the direct administration of cobra venom into the neoplasms.[268] The numerous investigations which followed on this question, and which have been summarized in special reviews,[269-271] may be divided into two categories,

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262. E. v. Balogh: Ann. d'anat. path. *10*: 65 (1933).
263. L. Ács & M. Dancsházy: Frankfurt. Ztschr. f. Path. *48*: 239 (1935).
264. H. G. Grünbaum & A. S. Grünbaum: Lancet *1*: 879 (1911).
265. Monaelesser & C. Taguet: Bull. Acad. de méd., Paris *109*: 371 (1933).
266. A. Calmette: Bull. Acad. de méd., Paris *109*: 375 (1933).
267. C. Taguet: Compt. rend. Acad. d. sc. *196*: 880 (1933).
268. A. Calmette, A. Saenz & L. Costil: Compt. rend. Acad. d. sc. *197*: 205 (1933).
269. Miffre: Contribution a l'étude du venin de cobra dans le traitment des tumeurs et des algies cancéreuses, Toulouse 1935.
270. D. v. Klobusitzky: Klin. Wchnschr. *16*: 569 (1937).
271. P. J. Hode & R. S. Thorne: Am. J. Roentgenol. *45*: 866 (1941).

one of which is devoted to the supposedly antiblastic effect of snake venoms, whereas the other deals with an analgetic action of these substances, so far as pains of cancer patients are concerned. Neither of these two trends of work led to undisputed results but, in general, little evidence has been furnished for a therapeutical usefulness of snake venoms in neoplasia. Although one author claims to have achieved improvements and even cures by means of this treatment,[272] these favorable results have not been obtained by the great majority of clinicians who employed this method. Moreover, the results of animal experimentation likewise did not support the assumption of a true antiblastic action of snake venoms, since tumors were found to be affected only by intratumoral administration, a procedure which cannot be considered very convincing since it most probably represents merely a necrotizing effect of highly toxic substances, and other ways of administration have been found to be entirely ineffective.[274-277] A snake venom (bothrops jararaca) did not specifically influence neoplastic tissue cultures, and injection of the substance into mice bearing Ehrlich carcinoma inhibited the tumor growth only when such large doses were employed that fifty per cent of the animals succumbed to the toxic action.[278] The venom of an adder was reported to exert some curative effects on guinea pig liposarcomas, but failed to act in a similar manner on tar cancer of mice.[279] Likewise, an-

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272. M. Meneghetti: Rev. radiol. clin. *3*: 828 (1934); abstr. Ztschr. f. Krebsforsch. *42*: Ref. 133 (1935).

273. J. Vellard, O. Penna & M. Vianna: Compt. rend. Acad. d. sc. *198*: 502 (1934).

274. E. Grasset & M. des Ligneris: Compt. rend. Soc. de biol. *116*: 386 (1934).

275. H. W. Julius: Acta brev. neerl. physiol. *5*: 49 (1935).

276. T. J. Kurotchkin & J. W. Spies: Proc. Soc. Exper. Biol. & Med. *32*: 1408 (1935).

277. A. Bessemans & L. Asaert: Ann. Inst. Pasteur *58*: 247 (1937).

278. B. Lustig & E. Werber: Ztschr. f. Krebsforsch. *43*: 359 (1936).

279. G. Bobeau: Rev. de path. comparée *36*: 1017 (1936).

other worker obtained negative results from the administering of cobra venom to mice with tar cancer.[280] While, thus, no rational basis has been presented for the employment of snake venoms as a true curative agent in neoplasia, these substances have won some recognition as helpful measures in palliative tumor therapy, namely as analgetic remedies in severe cancer pains. Such reports have been published by many authors.[271, 281–290] The pharmacological action of cobra venom was analyzed in a study, according to which a marked analgetic effect could be obtained in sixty-five per cent of patients, even when suffering from highly intractable cancer, treated with this substance. A central action of cobra venom was assumed,[291] and this view may be linked to the observation that brain tissue cultures showed themselves to be most sensitive to snake venom.[278] These findings make it probable that the neurotoxic constituent of snake venom is responsible for its analgetic property and, at the same time, it is obvious that the effects of the relief from pain are by no means confined to neoplasia, but will also be observed in pains originating from various other pathologies. But reference must also be made to divergent opinions expressed by some authors on

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280. K. A. Vannfält: Upsala läkaref. forh. *42*: 315 (1936); abstr. Am. J. Cancer *36*: 466 (1939).
281. A. Orticoni: Presse méd. *42*: 112 (1934).
282. R. C. Nicolini: Bol. y trab. Soc. de cir. de Buenos Aires *18*: 608 (1934); abstr. Am. J. Cancer *22*: 922 (1934).
283. H. A. Calderón: Bol. y trab. Soc. de cir. de Buenos Aires *18*: 615 (1934); abstr. Am. J. Cancer *22*: 922 (1934); Bol. inst. med. exper. para el estud. y trat. d. cáncer *11*: 87 (1934).
284. M. Laignel-Lavastine & N. T. Koressios: Bull. et mém. Soc. med. hôp. Paris *50*: 487 (1934).
285. J. Körbler: Klin. Wchnschr. *13*: 1185 (1934).
286. W. Zweig: Wien. klin. Wchnschr. *47*: 1140 (1934).
287. R. Dumatras: Rev. de path. comparée *35*: 700 (1935).
288. M. Kirschen: Wien. klin. Wchnschr. *49*: 648 (1936).
289. J. Nekula: Zentralbl. f. Gynäk. *60*: 328 (1936).
290. L. W. van Esveld: Biochem. Ztschr. *283*: 343 (1936).
291. D. I. Macht: Proc. Nat. Acad. Sc. *22*: 61 (1936); Ann. Int. Med. *11*: 1824 (1938).

the basis of their experiences with cobra venom treatment. The analgetic effect in cancer patients was seen to be only irregular and uncertain;[292, 293] or even was found completely lacking, in contrast to better results with arthritic pains.[294] It cannot yet be decided to what extent these discrepancies depended on differences in the preparations and dosages employed, and further investigations of this problem are desirable, since effective relief of pain in neoplasia, accomplished by other means than alkaloids, would represent an achievement not to be underestimated and might even—in an indirect manner—lead to a prolongation of life of the patients by increasing their resistance.

A close biological and pharmacological relationship may be supposed to exist between snake venoms and the venoms of bees. The latter substance was reported to cause a slight inhibition of tar cancer of rabbits,[295, 296] while clinical experiments with bee and wasp venom showed some analgetic action, but no influence on the tumor proper.[297] The meager experimental material does not warrant any definite conclusions, and the same applies to the proposal of a treatment of cancer with hydatid liquid.[298]

Some workers advanced one step further in therapeutical experiments in neoplasia by introducing living microorganisms into the body of tumor hosts. Here yeasts and lactacidogenic bacteria were principally employed since the underlying idea of these measures aimed at the accomplishing of metabolic changes in the neoplasms, in the sense that they should

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292. A. M. Dogliotti: 2nd Internat. Cancer Congress, Brussels *1*: 309 (1936).

293. M. des Ligneris & E. Grasset: Am. J. Cancer *26*: 512 (1936).

294. W. Löwenstein: Wien. klin. Wchnschr. *51*: 302 (1938).

295. G. Joannovics & X. Chahovitch: Bull. Acad. d. méd., Paris *107*: 892 (1932).

296. X. Chahovitch: 1st Internat. Cancer Congress, Madrid *1*: 84 (1933).

297. P. Natale: Tumori n. s. *9*: 324 (1935).

298. V. S. Perpiña & F. S. de Frutos: Zentralbl. f. Chir. *59*: 2408 (1932).

be deprived of needed carbohydrates by the action of the microorganisms. Saccharomyces suspensions have been administered, either directly into the tumor,[299] intravenously,[300] or perorally; [301, 302] some claimed success from this treatment, both in animal experiments and in human neoplasia, and injection of lactacidogenic bacteria was reported to result in a growth inhibition of the Ehrlich mouse carcinoma.[303]

Organotherapy. The category of cancer curative measures, covered in the following, is accessible only in a most limited extent to interpretations derived from general biological experiences, or from theoretical considerations. In most instances, empirically chosen substrates prepared from plants or animal organs have been tested for their antiblastic qualities, and the explanation of beneficial effects, insofar as they were observed, must be confined for the time being to the generality that they were due to an increase of the resistance of the body. Only few studies were concerned with plant substrates; here experiments with an extract of a Chinese plant (carcinolysin),[304] and with protein preparations derived from soy beans and various mushrooms [305] may be mentioned. Effects of these treatments, which were claimed to have been obtained in neoplasia, may be regarded as having been caused by a protoplasma activation occurring as a consequence of the administration of entirely heterologous protein substances.

The use of animal organs for tumor therapy took place most frequently in the form of administration of extracts of

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299. J. P. Nevorojskin: Vestn. Rentgenol. *15*: 335 (1935); abstr. Ztschr. f. Krebsforsch. *44*: Ref. 130 (1936).

300. G. H. Jacubson: Ztschr. f. Krebsforsch. *24*: 364 (1927).

301. O. Backofen & S. Ikeda: Ztschr. f. Krebsforsch. *39*: 308 (1933).

302. P. G. de Hoyos: Ist Internat. Cancer Congress, Madrid *1*: 475 (1933).

303. V. Carminati: Bol. istit. sieroterap, Milan. *12*: 205 (1933); abstr. Ztschr. f. Krebsforsch. *39*: Ref. 136 (1933).

304. T. Matsushita: Deutsche med. Wchnschr. *50*: 13 (1924).

305. W. Karo: Ztschr. f. Krebsforsch. *32*: 331 (1930).

several organs, inasmuch as these were believed, for various reasons, to contain substances capable of counteracting neoplastic growth. Along these lines, Maisin and his collaborators [306–308] developed a cancer therapy consisting of injections of extracts of brain, spleen, stomach and bone marrow, to which minute quantities of calcium, barium and strontium were added. Remarkable results have been reported from this treatment in animal tumors and also in tumor patients, especially in skin cancer, although it must be noted that radiation therapy frequently supported the organotherapy. The lability of the extracts employed was stressed by the authors, and more recently they devised methods for obtaining effective antiblastic extracts from pig-brain by rapid desiccation of the fresh tissue. The preparation obtained markedly lowered the tumor incidence in mice which had been treated with benzpyrene.[309] Another preparation of organotherapy, called I B 5, extracted from the duodenum, pancreas, liver, and spleen, has been recommended by Blumenthal and Jacobs.[310–312] These authors reported encouraging results from this therapy in animal experiments, and peroral administration of I B 5 to cancer patients was followed by beneficial effects. Similar experiences with I B 5 in human neoplasia were announced by other workers,[313, 314] but in one study in which this treatment was applied to sixty-two cancer cases, its

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306. J. Maisin, H. Vassiliadis & E. Picard: Bull. Assoc. franç. p. l'étude du cancer *21*: 255 (1932).
307. J. Maisin: 1st Internat. Cancer Congress, Madrid *1*: 481 (1933).
308. J. Maisin & Y Pourbaix: 2nd Internat. Cancer Congress, Brussels *1*: 389 (1936).
309. J. Maisin, Y. Pourbaix & J. Camerman: Compt. rend. Soc. de biol. *128*: 806 (1938).
310. F. Blumenthal: 1st Internat. Cancer Congress, Madrid *1*: 744 (1933).
311. E. Jacobs: Ist Internat. Cancer Congress, Madrid *1*: 749 (1933).
312. E. Jacobs: Ztschr. f. Krebsforsch. *38*: 545 (1933).
313. G. Ollino: Acta Cancrol. *1*: 43 (1934).
314. G. Picot & F. Cailliau: Bull. Assoc. franç. p. l'étude du cancer *24*: 173 (1935).

effect was limited to subjective improvements in some instances, but no definite influence on tumor growth was observed and metastases or recurrences occurred in seven patients during the treatment.[315] Later, Blumenthal and Jacobs [316, 317] announced that their continued work with organotherapy had led to the development of a new preparation, called "Aristotrop," which was claimed to represent a highly effective means of cancer therapy. A number of case histories were presented, allegedly proving complete cures of human neoplasms, accomplished solely by injections of Aristotrop, and it was reported that transplanted rat tumors showed marked histologic changes following treatment of the animals with this substance.[318] However, one of the authors of the latter communication later found himself obliged to retract his earlier statements, since repeated experiments did not demonstrate any curative effect of Aristotrop in animal experiments or in cancer patients.[319] Likewise, clinical experience, collected by numerous investigators with Aristotrop treatments—many of which have not been published but have come to the writers' attention by means of personal communications—stood in sharp contrast to the striking reports of the original authors. With the exception of one paper, in which an ostensible cure of one cancer patient was reported,[320] other workers found, at best, a general improvement of the patients' condition as a result of the treatment, whereas the neoplastic process was not influenced at all,[321]

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315. H. Cramer: München. med. Wchnschr. *84*: 572 (1937).
316. F. Blumenthal, E. Jacobs & H. Rosenberg: Schweiz. med. Wchnschr. *66*: 640 (1936).
317. F. Blumenthal: 2nd Internat. Cancer Congress, Brussels *2*: 379 (1936).
318. F. Cailliau & E. Jacobs: Paris méd. *2*: 173 (1936).
319. F. Cailliau: Bull. Assoc. franç. p. l'étude du cancer *27*: 6 (1938).
320. L. Conitzer: Wien. med. Wchnschr. *87*: 1260 (1937).
321. A. Simons: Schweiz. med. Wchnschr. *67*: 739 (1937).

or they declared Aristotrop to be completely useless.[322, 323]

A number of organo-therapeutic studies have been undertaken with the definite aim of counteracting neoplasia by an activation of the reticulo-endothelial function, and these investigations will accordingly be detailed in the next part of this chapter. In this connection, some results may be mentioned which have been obtained by products derived from mesenchymal tissue. Connective tissue of normal persons was found to contain a substance which inhibited the growth of transplanted rat tumors,[324] and cures of rat tumors were reported to have resulted from injections of muscle hydrolysates[325] (cf. chapter IX, part 2).

It is comprehensible that work with extracts of organs suggested the possibility that endocrine factors of known or unknown nature might be involved in the effects observed, and such aspects may be ascribed in particular to the following experiments: The property of inhibiting tumor growth has been attributed to an adrenal extract to which phenol has been added, and which was transformed into a "red solution" by the action of oxidases present in the extract.[326] Other authors assumed that bone marrow would exert a regulatory influence on growth, and reported that they had demonstrated an abnormal condition of this tissue in neoplasia as to its potassium content (cf. chapter I). Accordingly, they tried to implant normal bone marrow into cancer patients and reported beneficial effects from this treatment;[327] however,

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[322] M. Brögli: Schweiz. med. Wchnschr. *67*: 95 (1937).

[323] M. Fořt: Časop. lék. česk. *75*: 1683 (1936)

[324] J. J. Morton & D. N. Beers: J. Exper. Med. *61*: 59 (1935).

[325] A. H. Roffo: Bull. Assoc. franç. p. l'étude du cancer *26*: 602 (1937); Lancet *2*: 184 (1938); Bol. inst. med. exper. para el estud. y trat. d. cáncer *14*: 257 (1937).

[326] H. Auler, H. Schlottmann, W. Rubenow, P. Meyer & B. Wolff: Ztschr. f. Krebsforsch. *32*: 195 (1930).

[327] P. Rosenstein & H. Köhler: Med. Klin. *27*: 1095 (1931); Deutsche med. Wchnschr. *58*: 1112 (1932).

various extracts of calf bone marrow were found to be without any influence on the Ehrlich mouse carcinoma.[328] A combined therapy, closely related to endocrine aspects, has been proposed, which consists of injections of extracts of thymus, adrenals and gonads, plus extracts of the organ which is affected by neoplasia in the patient to be treated,[329] but no experiences with this therapy have been published. It must be stressed that a true endocrine nature of the experiments mentioned cannot at present be accepted on account of the lacking biological or chemical proof and assay for hormone factors in the substrates employed. Moreover, substances influencing normal and neoplastic growth have been shown to be present in a great variety of tissues; these findings will be surveyed in the second part of chapter IX.

5. *Reticulo-Endothelial System.*

Whatever the final evaluation derived from continued work may be, which will be applied to the findings presented here, it cannot be denied that the concept ascribing a decisive part in neoplasia to the reticulo-endothelial system has served as a very useful working hypothesis giving rise not only to significant theoretical considerations, but also to some valuable starting points for achieving practical aims. It may to some extent be necessary to justify the reason for including a discussion of the reticulo-endothelial system, a topic of predominantly morphological background, in this treatise on biochemical cancer research. Yet, in going back to the original discovery and definition of the reticulo-endothelial system by Aschoff, it will be recognized that this cell apparatus, originally comprising the Kupffer cells of the liver, the sinus cells of the spleen, the endothelia of vessels, and some histological structures in lymph nodes, adrenals and pituitary, has been demonstrated primarily by a functional quality, i.e., by its ability to store selectively certain physiological, or artificially introduced colloids. This concept has since found recognition from the majority of investigators, although continued elaboration tended to expand the boundaries of this sytem. Aschoff,[330] also acknowledged later, in an exhaustive presentation of the subject, that one is warranted in assuming the existence of an enlarged reticulo-endothelial system which includes variable elements of the entire mesenchyme (activated mesenchyme, histiocytic apparatus). Hence it is apparent that this system hardly constitutes an anatomical or histological entity, but must rather be regarded as being defined by functional qualities, with which varying parts of the mesenchyme may be endowed in consequence of physiological or pathological processes or experimental interferences. Moreover, a great many clinical observations and experimental findings clearly indicated that, in addition to the ability to store colloids, the reticulo-endothelial system also participates physiologically and pathologically in immunological phenomena, and is connected with chemical, physico-chemical and metabolic processes. In view of these complex relations, it is deemed necessary to stress the following factors as requirements for a correct interpretation and evaluation of studies performed in this vast field. One, the fact that the reticulo-endothelial system is scattered throughout the whole body, and is subject to variations of its extent and its functional activity; two, experimental or pathological damages of this system, which are expressed in an impairment of one or more of its functions, do not necessarily preclude that other functions persist unimpaired, and in general the high regenerative capacity of

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[328] M. Fořt: Časop. lék. česk. *75*: 46 (1936)

[329] H. H. Vassileff: 1st Internat. Cancer Congress, Madrid *2*: 128 (1933).

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330. L. Aschoff: Ergebn. d. inn. Med. u. Kinderh. *26*: 119 (1925).

reticulo-endothelial cells must always be taken into consideration; three, although spleen represents the largest concentration of reticulo-endothelial elements in one organ, it does not appear permissible generally to identify effects, resulting from interferences with this organ, as being entirely dependent on reticulo-endothelial factors, since spleen also contains large quantities of other cellular structures which serve different purposes. Especially as far as investigations of the reticulo-endothelial system in connection with neoplasia are concerned, neglect of the factors mentioned may have been the cause of many discrepancies. The arrangement of the following material is based on the plan of dividing the studies on the reticulo-endothelial system in neoplasia into four categories. One, examinations of the morphological status in tumor hosts, two, investigations of the functional activity, three, experimental interferences which affect the tumor process either by impairing or by activating the reticulo-endothelial system, the latter aiming at therapeutical effects; four, discussion of findings which correlate the various biochemical and metabolic phenomena observed in neoplasia to alterations of the reticulo-endothelial system.

Morphological findings. Special interest is attached to investigations of this kind, since they may be able to furnish a better insight into the theoretical deductions and experimental results, to be mentioned later. A few years after Aschoff's first publication on the reticulo-endothelial system, it was reported that animals exhibited a hypertrophy of reticulo-endothelial elements in the spleen and liver shortly following tumor inoculation, a phenomenon interpreted as a defensive reaction. On the other hand, autopsy studies revealed an atrophy of reticulo-endothelial elements in liver, spleen and lymph nodes of persons deceased of malignant tumors, and these findings were regarded as indicating a predisposition for metastases formation in the organs mentioned.[331] In the writers' opinion, the different behavior of the reticulo-endothelial system in clinical and experimental neoplasia may be explained by the fact that in the latter case a malignant tumor is suddenly transferred to an intact organism, which is still capable of mobilizing defensive reactions whereas, in the former instance, the spontaneous tumor development obviously takes place in a body with deteriorated protective mechanisms. More recently, transplantation of tumors was also found to be followed by a hypertrophy of reticulo-endothelial elements, particularly by a proliferation of histiocytes and Kupffer cells in the liver, and by reticular hyperplasia of the spleen.[332, 333] Significantly, the hyperplasia of the reticulo-histiocytic apparatus has been observed to persist in mice which had acquired a resistance against transplantable tumors.[334] It is noteworthy that certain dietary measures (disturbed equilibrium of vitamins, cf. chapter V), which led to spontaneous tumor formation in animals, have been reported to be accompanied by an impairment of the mesenchyme and especially of the reticulo-endothelial system, as evidenced by histological examination.[335] Interesting findings have also been obtained with chemically induced tumors; tar treatment of mice [336] and of rabbits [337] resulted in a different reaction of those animals which eventually contracted malignancy and of those which remained refractory. The former exhibited a marked follicular aplasia of the spleen—and similar histological findings were seen in spleens of tumor patients [336]—whereas refractory animals showed a

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331. V. Neudörfer: Wien. klin. Wchnschr. *31*: 803 (1918).
332. B. Borghi: Tumori n. s. *1*: 15 (1928).
333. A. Calò: Ztschr. f. Krebsforsch. *37*: 151 (1932).
334. M. Volterra: Ztschr. f. Krebsforsch. *35*: 315 (1932).
335. E. Müller: Ztschr. f. Krebsforsch. *34*: 10 (1931).
336. G. Schorr & S. Damberg: Vestn. Rentgenol. *8*: 8; 11 (1930); abstr. Ztschr. f. Krebsforsch. *33*: Ref. 4 (1931).
337. A. Babes: Bull. Assoc. franç. p. l'étude du cancer *19*: 232 (1930).

hyperplasia of follicles and of reticulo-endothelial elements. An extensive amyloidosis has been found in the spleen, liver and kidneys of mice treated with benzpyrene, and these alterations, obviously implying a severe damage of reticulo-endothelial cells, significantly preceded the papilloma and tumor formation, for which reason the authors supposed a causal connection to exist between these effects and the subsequent neoplastic growth.[338] Furthermore, it was stated that roentgen irradiation, and likewise tar treatment, induced irreversible alterations of the entire mesenchyme in the animals, prior to tumor development: using colloidchemical terms, the author defined these changes as a transformation from the sol phase into the gel phase.[339] Since this structural deviation may strongly disturb the passage of substances between cellular and humoral media, it is comprehensible that a severe functional insufficiency of the cells may result, thus preventing, for instance, the storing of colloids present in the body fluids.

In addition to these histological findings, the gross anatomy of the spleen has been found to deviate from the normal, inasmuch as a hypertrophy, resulting in an increased weight of the organ, has very regularly been observed in animals bearing various types of neoplasms.[340, 341] These findings have been confirmed by numerous investigations which studied the Brown-Pearce rabbit carcinoma,[342] Rous sarcoma of fowl,[343] Flexner-Jobling rat carcinoma,[344] Jensen rat sarcoma,[345]

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338. X. Chahovitch, M. Vichnjitch & J. Ignjatchev: Libro de Oro, Buenos Aires, p. 206 (1935).

339. E. Zurhelle: Med. Klin. *33*: 1595 (1937).

340. A. H. Roffo: Ztschr. f. Krebsforsch. *30*: 180 (1929); Bol. inst. med. exper. para el estud. y trat. d. cáncer *5*: 383 (1928).

341. D. C. A. Butts: Libro de Oro, Buenos Aires, p. 179 (1935).

342. W. H. Brown & L. Pearce: Proc. Soc. Exper. Biol. & Med. *20*: 472 (1932).

343. F. Kobliha: Ztschr. f. Krebsforsch. *32*: 344 (1930).

344. Y. Okamoto: Gann *21*: 31 (1927).

345. J. M. Twort & M. Lasnitzki: Endocrinology *23*: 87 (1938).

Walker carcino-sarcoma 256 of rats[346] and spontaneous tumors of rats.[347] Similar relations were also noticed in animals with chemically induced tumors: tar treatment of mice caused a spleen enlargement in the animals, prior to the development of malignancy;[348] the administration of tar, dibenzanthracene, benzpyrene or methylcholanthrene to mice was reported to be followed by a spleen enlargement which was approximately proportional to the potency of the carcinogen employed;[349] and butter yellow feeding caused a spleen hypertrophy in rats soon after the start of the experiment.[350] Although the mechanism of the spleen hypertrophy in tumor-bearing animals has by no means been clarified, and at least in certain instances unspecific factors—secondary infection of the neoplasms—may be involved, an interesting clue has been furnished by the finding that a spleen hyperplasia could also be induced in animals by means of injections of tumor extracts, particularly of lipids, and by injections of blood derived from animals or humans affected with malignancy.[340] However, this effect does not seem to be limited to neoplasia, since similar changes of the spleen were seen in animals injected with blood or the urine of pregnant women. Accordingly, a common mechanism, affecting the spleen, was assumed to be present in neoplasia and in pregnancy, inasmuch as both conditions are accompanied by an increased lipid metabolism, resulting in an augmented functional activity of the spleen.[351] On the other hand, a connection between the resistance against neoplasia and the size of the

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346. B. Sure, R. M. Theis, R. T. Harrelson & L. Farber: Am. J. Cancer *36*: 252 (1939).
347. K. A. Winter: Ztschr. f. Krebsforsch. *40*: 410 (1934).
348. N. Waterman: Frankfurt. Ztschr. f. Path. *44*: 540 (1933).
349. J. M. Twort & C. C. Twort: Am. J. Cancer *28*: 78 (1937); *35*: 80 (1939).
350. R. Kinosita: Yale J. Biol. & Med. *12*: 287 (1940).
351. A. H. Roffo: Bol. inst. med. exper. para el estud. y trat. d. cáncer *10*: 651; 745 (1933).

spleen, is suggested by the report that rabbits, refractory against inoculation of Brown-Pearce carcinoma, exhibited a striking splenic enlargement.[352] Another tissue, containing reticulo-endothelial elements, viz., the bone marrow, was found hypertrophied to a varying degree in tumor-bearing animals, but this phenomenon was interpreted as being of a secondary nature, that is, as being due to resorption of necrotic tumor material.[353]

Among structural peculiarities of the reticulo-endothelial system in tumor pathology, research workers have for a long time, been concerned with the comparative rarity of spleen metastases of malignant tumors, a fact which is the more striking in view of the rich supply this organ has of blood and lymph vessels, and its anatomical proximity to intestinal organs frequently affected with malignancy. Accordingly, an antiblastic quality of spleen tissue has been inferred from these findings, but it is doubtful whether such a property can be regarded as being dependent on the reticulo-endothelial elements present in the spleen; for other tissues of partly reticulo-endothelial character, such as lymph nodes and bone marrow, are by no means protected against the formation of metastases. Moreover, other explanations have been offered for the rare incidence of spleen metastases, such as peculiar arrangements of lymph vessels in this organ,[354] or large accumulations of enzymes originating from disintegrating leucocytes.[355, 356] Yet, in a more recent study in which this problem was reconsidered by reviewing human tumor pathology, and by investigating mice with Ehrlich carcinomas, it was stressed that microscopic spleen metastases were found

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352. P. Kallós & L. Kallós-Deffner: Schweiz. Ztschr. f. allg. Path. *3*: 149 (1940).
353. J. W. Orr: J. Path. & Bact. *42*: 105 (1936); *45*: 579 (1937).
354. D. E. Dial: Am. J. Path. *6*: 79 (1930).
355. S. S. Chalatow: Virchow's Arch. f. Path. *217*: 140 (1914).
356. H. Spude: Ztschr. f. Krebsforsch. *21*: 294 (1924).

in ten per cent of the animals, whereas macroscopic metastases were extremely rare; hence it was concluded that spleen represents a very unfavorable medium for neoplastic growth.[357] Likewise, the result of 580 autopsies of cancer patients, showing spleen metastases in only 3.2 per cent, has been interpreted as supporting the assumption of special antiblastic properties of the spleen.[358] No uniform conclusions have been drawn from animal experiments intended to clarify this problem; some authors found normal growth of tumors inoculated into spleen,[359] or of neoplastic tissue cultures brought into contact with splenic tissue,[360] but more recently a failure of intrasplenic implantation of Brown-Pearce rabbit carcinoma was reported,[361] and local implantations of carcinogenic hydrocarbons into liver and spleen showed these tissues to be the least susceptible for tumor development as compared with other organs.[362]

An interesting question has come under consideration in a few studies devoted to the demonstration of reticulo-endothelial tissue within the neoplasms proper,[363, 364] but no conclusive evidence has as yet been furnished as to whether a reticulo-endothelial activity—perhaps of a compensatory nature—is exerted by tumors. An affirmative answer to this question might be derived from the observation according to which tumor-bearing rats tolerated splenectomy better than tumor-free animals,[365] but doubtless a more thorough analysis of this phenomenon is required before it can be definitely evaluated.

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[357] P. Czigler: Acta Cancrol. *2*: 47 (1936).

[358] L. I. Flantchik: Problems of Oncology, Kharkov *10*: 38 (1936); abstr. Am. J. Cancer *31*: 152 (1937).

[359] E. O. Tatarinow & R. A. Dimschitz: abstr. Ztschr. f..Krebsforsch. *44*: Ref. 73 (1936).

[360] H. N. Stevenson: Proc. N. Y. Path. Soc. *16*: 60 (1916); J. Cancer Res. *2*: 245; 449 (1917).

[361] P. Kallós & L. Kallós-Deffner: Schweiz. Ztschr. f. allg. Path. *3*: 11 (1940).

[362] M. J. Shear, H. L. Stewart & A. M. Seligman: J. Nat. Cancer Inst. *1*: 291 (1940).

[363] G. A. Chiurco & A. Volpe: Ztschr. f. Krebsforsch. *38*: 113 (1932).

[364] I. Costero: Arch. españ. oncol. *3*: 71 (1932); abstr. Ztschr. f. Krebsforsch. *39*: Ref. 10 (1933).

Summarizing the morphological findings obtained in neoplasia, it must be stated that the available material is as yet insufficient for arriving at final conclusions. The clarification of this problem is obviously rendered difficult by the fact that histological methods frequently fail to facilitate a precise insight into the status of this system, unless its cells have been given opportunity to store colloids, which have been artificially introduced into the body. So far as human pathology is concerned, the injection of harmless colloids into cancer patients ante exitum, as was done some time ago for other purposes,[366] may facilitate a subsequent histological examination of the reticulo-endothelial elements in these patients. Furthermore, attention has recently been called to a necessary revision of views, which concern the function of the adiposal tissue in the body. It has been stressed that this part of the mesenchyme should be regarded "not merely as a storehouse," since evidence was presented, inferring that this tissue fulfills specific functions in physiology and pathology.[367] By adopting this view it may be possible to regard the depletion of the adiposal tissue which occurs in neoplastic waste, not solely as being caused by nutritional factors, but as a morphological expression of a functional impairment of this part of the mesenchyme. As to animal experimentation, investigations on the reticulo-endothelial morphology should be extended to animals belonging to strains with a high susceptibility to spontaneous neoplasia, so as to ascertain whether or not the formation of these tumors is preceded or accompanied by morphological alterations of the reticulo-endothelial system.

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[365] H. Hirschfeld & F. P. Tinozzi: Ztschr. f. Krebsforsch. *26*: 304 (1928).

[366] H. Eppinger & F. Stöhr: Klin. Wchnschr. *1*: 1543 (1922).

[367] G. H. Wells: J. A. M. A. *114*: 2177; 2284 (1940).

Functional activity. Of the numerous physiological functions ascribed to the reticulo-endothelial system, so far only one has served as a basis for tests devised for analyzing the functional activity of this system, i.e., the storing of colloids. At the same time, this measure, as remarked above, has been utilized for histological examinations and hence the close connection between structural and functional qualities of reticulo-endothelial elements appears supported once more. Findings of an older date have been confirmed, inasmuch as by means of storing with colloids a hypertrophy of reticulo-endothelial elements was noticed in animals during the first stages of tumor inoculation.[368] This obviously may be regarded as a defensive reaction, whereas the observation that in animals treated with tar the carmine-storing ability of reticulo-endothelial cells is markedly diminished even in the precancerous phase[369] indicates a functional insufficiency induced by the carcinogen. Likewise, it was reported that tumor-bearing mice fail to respond to short-wave irradiation with a stimulation of their mesenchymal activity, as was observed in tumor-free animals by means of trypan blue loading of the histiocytes, and this finding infers a depressed functional state of the reticulo-endothelial system in tumor hosts.[370] Rats injected with benzpyrene exhibited a diminished capacity to store carmine in the reticulo-endothelial elements of spleen and liver, and this impairment was found to run parallel to the number of injections of the carcinogen applied, and to the tumor development.[371] Yet, while these investigations employing histological methods necessitated the sacrifice of the experimental animals, and hence are not applicable to clinical studies, other methods have been devised which permit a functional analysis of the reticulo-endothelial system in vivo and, accordingly, they must be considered to be more suitable for an elucidation of this problem. The principle of these tests is based on the assumption that a colloidal dye solution, injected intravenously, will be partly absorbed by reticulo-endothelial elements, and that a decrease of the dye concentration in the blood, determined by dye estimations in the blood at different time intervals following the injection, may permit the gaining of information as to the functional activity of the reticulo-endothelial system. The most frequently used test of this kind is the Congo Red method, developed by Adler and Reimann.[372] A modification of this test has been proposed, which, it was believed, would be preferable to the original procedure on account of the smaller quantities of blood required, and on account of a more exact colorimetric estimation of the dye concentration.[373] According to the original investigation, forty to seventy per cent of the Congo Red present in a sample withdrawn from normal persons six minutes after the injection of the dye, could be detected in the blood sixty minutes after the injection. This finding has been expressed as a "Congo Red index" of forty to seventy per cent, and was believed to indicate that the rest of the dye had been absorbed by reticulo-endothelial elements. By this method, a severe damage of the reticulo-endothelial function has been ascertained as existing in acute infections, e.g., in pneumonia, since only a smaller amount of Congo Red was stored by the reticulo-endothelial system under these conditions (Congo Red index higher than seventy per cent). Significantly, Adler and Reimann also found a high Congo Red index in two cancer patients, and subsequently several reports, though based on a comparatively small number of patients tested, substantiated the assumption that the reticulo-endothelial function, as expressed by the Congo Red index, is markedly depressed in neoplasia.[374-378] By means of the modified Congo Red test,[378] a damage of the reticulo-endothelial system could be demonstrated in fourteen out of fifteen tumor patients tested, whereas normal Congo Red indices were obtained in twenty-seven out of thirty-nine patients clinically free of malignancy. In six out of the twelve cases, in which an impairment of the reticulo-endothelial function was found in the absence of neoplasia, a definite cause (infection, lead poisoning, etc.) could be detected, which explained this alteration.[379] In a larger number of cases, the modified Congo Red test has recently been employed with the following results. In forty-nine persons, clinically free of malignant tumors, Congo Red indices were found ranging between forty and seventy per cent, whereas values over seventy per cent were obtained in eighty-six out of one hundred tumor patients. While thus the fact of an impaired reticulo-endothelial function in neoplasia appears to have been clearly established, these findings cannot be directly utilized for diagnostic purposes, since damages of this system are known to occur and have been demonstrated by means of the Congo Red test in a number of other pathologies, particularly in infections. However, in addition to the theoretical significance which may be attached

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[368] B. Borghi: Tumori n. s. *3*: 289 (1929).

[369] M. Oike: Jap. J. Obst. & Gynec. *14*: 438 (1931).

[370] K. P. Markuse, D. A. Losinsky & N. Malow: Ztschr. f. Krebsforsch. *44*: 415 (1936).

[371] C. Koga: Scripta Soc. radiol. jap. *5*: 405 (1937); abstr. Ztschr. f. Krebsforsch. *47*: Ref. 209 (1938).

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[372] H. Adler & F. Reimann: Ztschr. f. d. ges. exper. Med. *47*: 617 (1925).

[373] K. Stern: Wien. klin. Wchnschr. *50*: 1579 (1937).

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374. U. Krätschell: Fortschr. d. Med. *51*: 983 (1933).
375. S. Barbera: Bol. soc. med.-chir. Catania *3*: 98 (1935); abstr. Ztschr. f. Krebsforsch. *42*: Ref. 82 (1935).
376. M. Battiloro: Ann. ital. chir. *15*: 783 (1936).
377. U. Borghetti: Arch. ital. chir. *50*: 532 (1938).
378. W. Schroeder: Klin. Wchnschr. *18*: 248 (1939).
379. F. Haas: unpublished study; personal communication.

to the results mentioned, some support has been furnished for the possibility of deriving a prognostic evaluation from the degree of the reticulo-endothelial insufficiency as expressed in the Congo Red index, and of estimating to some extent the effectiveness of therapeutic measures by means of tests performed repeatedly during and after the treatment (radiation therapy).[380]

Similar conclusions as to a correlation between neoplasia and reticulo-endothelial activity have been derived from animal experiments by employing the Congo Red test. A decreased reticulo-endothelial function was found in rabbits with Kato sarcoma[381] and analogous results were obtained in studies with rabbits inoculated with Brown-Pearce carcinoma.[382] Furthermore, it was reported that painting with tar, administration of dibenzanthracene or inoculation of Brown-Pearce carcinoma lowered the reticulo-endothelial function, and this mesenchymal anergy was thought to constitute an important factor in carcinogenesis.[383] Rabbits have been repeatedly injected with dibenzanthracene for several months, and this treatment resulted in a considerable depression of the reticulo-endothelial function in thirteen out of fourteen animals.[384] A similar effect was obtained by administering benzpyrene and methylcholanthrene to rabbits,[385] or by subjecting the animals to protracted roentgen irradiations,[386] but it is extremely significant that a treatment with non-carcinogenic hydrocarbons (1, 2-benzanthracene, phenanthrene, anthracene) in general failed to affect the reticulo-endothelial activity.[386] Hence the effects observed were probably not due merely to the toxic qualities of the substances employed, but were directly connected with the carcinogenic quality.

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[380] K. Stern: J. Lab. & Clin. Med. *26*: 809 (1941).

[381] T. Kageyama: Jap. J. Obst. & Gynec. *20*: 291 (1937).

[382] K. Stern: unpublished study.

[383] R. E. Kavetsky: Bull. Biol. et Méd. exper. URSS *3*: 378 (1937); Acta med. URSS *2*: 74 (1939).

[384] T. Csato, C. Wetzler-Ligeti & B. P. Wiesner: Am. J, Cancer *36*: 257 (1939).

[385] C. Hoch-Ligeti: Am. J. Cancer *39*: 365 (1940).

[386] C. Hoch-Ligeti: Cancer Research *1*: 28 (1941).

It must, however, be admitted that objections have been raised against the value of the Congo Red test for assaying the reticulo-endothelial function, inasmuch as histological examinations failed to give definite proof that this dye is taken up by reticulo-endothelial cells.[387, 388] On the other hand, ample experimental evidence is available to confirm the fact that artificial impairments of the reticulo-endothelial system (splenectomy, blockade) is followed by changes of the Congo Red index in a sense which indicates a reticulo-endothelial insufficiency. The possibility may be considered as to whether the removal of Congo Red from the blood may be indirectly influenced by the functional state of the reticulo-endothelial system, without direct storage of the dye in the reticulo-endothelial cells. Certainly, it would appear highly desirable to devise other functional tests for the reticulo-endothelial activity, depending on immunological, physico-chemical or metabolic mechanisms, particularly, because different functions of the system might be impaired to a varying extent in certain pathologies. Yet, so far such supplementary tests have not been made available, or are of a too complex nature to permit their clinical application.[cf. 389]

Influence of the reticulo-endothelial system on the tumor process. While the preceding investigations have been devoted to the demonstration of reticulo-endothelial insufficiency in neoplasia and, for the greater part, could not give an answer to the question as to whether this phenomenon

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387. E. Letterer: Ztschr. f. d. ges. exper. Med. *93*: 89; 103 (1934).

388. A. P. Richardson: Am. J. M. Sc. *198*: 82 (1939).

389. H. Brumann: Methoden zur Untersuchung des R. E. S.; Abderhalden's Arbeitsmethoden, Abtl. 8, Tl. 1, No. 7, Lief. 383.

precedes tumor formation or results from it, this important problem appears to have been brought nearer to a solution by a great number of experiments which are described in the following survey. One of the approaches serving this purpose consisted of studying tumor formation and growth in animals the reticulo-endothelial system of which had been damaged, and the prevailing majority of such investigations arrived at positive results, i.e., a stimulation of tumor development and growth in consequence of reticulo-endothelial insufficiency has been established. The damages experimentally induced have been chiefly either splenectomy, i.e., a quantitative reduction of the reticulo-endothelial system, or the so-called blockade, viz., a functional impairment of reticulo-endothelial elements, caused by loading them excessively with certain colloids. In this manner splenectomy has been frequently reported as stimulating the growth of inoculated tumors,[358, 390-393] and similar results were obtained when tumor-bearing animals were injected with india ink,[363, 394] trypan blue,[395, 396] carmine or collargol.[397] Formation of metastases appeared to be enhanced following blockade of the animals,[374] and sub-threshold doses of tumor suspensions resulted in neoplastic growths when the reticulo-endothelial activity of mice had been impaired by blockade with india ink.[398] More recently it has been demonstrated that spontaneous mouse tumors, when grafted to mice of another

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390. G. Joannovics: Wien. Klin. Wchnschr. *29*: 345 (1916).
391. E. G. Oser & E. E. Pribram: Ztschr. f. exper. Path. u. Ther. *12*: 295 (1913).
392. B. E. Brüda; Wien. klin. Wchnschr. *42*: 166 (1929).
393. T. Reeke: München. med. Wchnschr. *77*: 1706 (1930).
394. V. Baroni & O. Comsia: Compt. rend. Soc. de biol. *111*: 1103 (1932).
395. G. O. E. Lignac & G. A. Kreuzwendedich von dem Borne: Nederl. tijdschr. f. geneesk. *71*: 1302 (1927).
396. Y. Imagawa: Tr. Jap. Path. Soc. *20*: 710 (1930).
397. J. R. Meyer: Ann. Fac. Med. Sao Paolo *4*: 191 (1930); abstr. Ztschr. f. Krebsforsch. *33*: Ref. 120 (1931).
398. E. Urbach & H. Schnitzler: Wien. klin. Wchnschr. *41*: 941 (1928).

strain, grew far better and faster if the animals had been previously injected with trypan blue, i.e., if the activity of their reticulo-endothelial system had been depressed.[399] Likewise, injection of thorotrast was found to stimulate the growth of Brown-Pearce rabbit carcinomas, but it was stressed that this effect was observed only when a smaller dose (1 cc.) was employed, whereas larger quantities (9 cc.) failed to promote tumor growth, probably because they had induced a compensatory reticulo-endothelial hyperplasia.[400] Similar observations were made with chemically induced tumors: development and growth of tumors due to coal tar, or to dibenzanthracene, were found accelerated and increased in splenectomized mice, as compared with the response of control animals.[401]

Some authors even arrived at results of more far reaching implications, inasmuch as it was believed that experimentally induced reticulo-endothelial insufficiency might remove the normally existing barrier of heterogenicity, and thus facilitate heterologous tumor transplantation. Mouse tumors were reported to grow when inoculated to splenectomized rats,[402] and a fowl sarcoma was found transplantable to mice, following blockade of the animals with iron saccharate.[403] Moreover, the possibility of producing tumors in animals with impaired reticulo-endothelial function by means of injections of cell-free filtrates of mammalian tumors has been claimed, a phenomenon which otherwise does not seem to be obtainable (cf. chapter IX, part 3). Thus tumor formation was reported as resulting from the injection of a rat carcinoma filtrate, admixed with "kieselgur" to splenectomized rats,[404] and extensive studies have been performed in mice injected with india ink; in eight per cent of these animals, tumor development was seen after the injection of ultrafiltrates of rat sarcomas, and histological examination proved the "filtrate tumors" to be identical with the original neoplasm.[405] The author of the previous report, furthermore, from histological examinations derived significant indications for an involvement of the reticulo-endothelial system in these phenomena; injection of the tumor filtrates was followed by an increase of the Kupffer cells in the liver and a proliferation of other reticulo-endothelial elements; these changes disappeared in animals in which tumor formations occurred, but persisted in refractory rats, and hence this reticulo-endothelial response was believed to represent the causative factor for the resistance of the animals against tumor growth.[406]

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[399] H. B. Andervont: Pub. Health Rep. *51*: 591 (1936).

[400] P. Brouwer: Beitr. z. klin. Chir. *168*: 616 (1939).

[401] F. M. Khaletzkaya: Arch. path. Anat. u. path. Physiol. (Russ.) *5*: 31 (1939); abstr. Am. J. Cancer *40*: 262 (1940).

[402] B. E. Brüda & H. Pfeiffer: Ztschr. f. d. ges. exper. Med. *68*: 116 (1929).

[403] G. Roskin: Ztschr. f. Krebsforsch. *24*: 122 (1927).

The aforementioned findings lead to the interesting problem of whether impairments of the reticulo-endothelial function may influence the experimentally changed resistance ("immunity") of animals against malignant tumors. A positive answer to this question was furnished in a study performed thirty years ago, in which splenectomy was found to inhibit the development of acquired immunity against transplanted tumors.[407] Divergent results obtained in a later investigation—in which blockade of animals failed to influence the development of tumor immunity[394]—might have been due to differences in the experimental arrangement. In fact, it has been definitely ascertained that "tumor immunity" produced by the injection of embryonic mouse skin into mice was nullified by subcutaneous injection of trypan blue,[408] and splenectomy was found to reduce the resistance of rabbits against inoculation of Brown-Pearce carcinoma.[852] Similarly, it was demonstrated that the growth of Shope rabbit papilloma was stimulated when the animals had been subjected to a protracted general roentgen irradiation, and this treatment was believed to have adversely affected the reticulo-endothelial activity.[409] Most likely a similar interpretation may be applied to the finding that susceptibility to, and growth of, inoculated tumors was increased in mice which had been treated with tar without tumor formation resulting.[410] As implied by reports mentioned above, tar administration causes a damage of the reticulo-endothelial tissue and, consequently, may reduce the resistance to tumors in a manner similar to the effects of splenectomy or blockade with colloids, or of prolonged roentgen irradiation. A report may also be mentioned, in this connection, according to which blockade of the reticulo-endothelial system with trypan blue in young rabbits, and simultaneous affliction of mechanical traumas to the brain, resulted in a formation of a glioma in one animal.[411] But in view of the scant number of experiments this effect cannot be regarded as having definitely been secured. Another means of damaging the reticulo-endothelial function has been devised in the form of a "cytotoxic antireticular serum," this serum being derived from horses injected with human liver pulp and with bone marrow, and the administration of this serum was found to decrease the resistance of animals against tumor transplantation.[412] Later, however, reports will be mentioned, which demonstrated the possibility of stimulating the reticulo-endothelial function by means of the same serum.

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[404] D. H. Morris: Proc. N. Y. Path. Soc. *16*: 186 (1916).
[405] Rh. Erdmann: Ztschr. f. Krebsforsch. *27*: 69 (1928).
[406] Rh. Erdmann: Ztschr. f. Krebsforsch. *33*: 189 (1931).
[407] H. Apolant: Ztschr. f. Immunitätsforsch. u. exper. Ther. *10*: 103 (1911).

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[408] R. J. Ludford: Brit. J. Exper. Path. *12*: 45; 108 (1931).

[409] J. Clemmesen: Am. J. Cancer *35*: 378 (1939).

[410] E. Puccinelli: Pathologica *21*: 333 (1929); abstr. Cancer Rev. *5*: 573 (1930).

[411] O. Marburg: Wien. klin. Wchnschr. *50*: 1509 (1937).

[412] A. A. Bogomolets: Med. zhur. *6*: 995 (1936); *8*: 301 (1938); Acta med. URSS *2*: 65 (1939).

Reticulo-endothelial therapy. It is easily comprehensible that the results enumerated in the preceding sections incited numerous authors to exert efforts to utilize for therapeutical purposes the apparently close relationship between reticulo-endothelial function and tumor pathology; that is, to increase the reticulo-endothelial activity in the hope of thus effectively counteracting neoplasia. The great number of attempts in this direction may be classified into three groups, according to the underlying idea which decisively influenced their practical execution. The first category may be called a substitution therapy, inasmuch as reticulo-endothelial organs, or extracts from them have been employed for the purpose of compensating the reticulo-endothelial insufficiency in neoplasia; the second group may be termed as stimulation therapy, and comprises endeavors to activate the mesenchymal tissue by means of various biological stimuli. Finally, a third class of studies is devoted to accomplishing an activation of the reticulo-endothelial system by employing definite chemical substances, and hence may be regarded as an attempt to create a specific pharmacology of the reticulo-endothelial system.

Substitution therapy. It must be anticipated that this form of reticulo-endothelial therapy will be encumbered with two basic difficulties. On the one hand, the basic theoretic premise —the production by the tissue of an exocrine or endocrine factor, which were to be replaced by the substitution therapy —has by no means been established for the reticulo-endothelial system; on the other hand, the practical side of the problem is complicated by the fact that reticulo-endothelial elements, from which the desired preparation should be derived, are scattered throughout the whole body; and even in the spleen, where their greatest concentration is to be found, tissues of another nature and of different functional qualities are also present. These difficulties may perhaps account for the fact that this trend of work has not so far met with ultimate success, in spite of some encouraging results observed principally in animal experiments.

The first investigations to be discussed were undertaken prior to the establishment of the theoretical concept of the reticulo-endothelial system, and were initiated because of a supposed antiblastic quality of spleen tissue. As far back as 1910, clinical experiments were performed by suturing fresh beef spleen to inoperable tumors, and crater-shaped defects in the neoplasms were reported to result from this procedure.[413] By means of animal experimentation, Woglom [414] and Braunstein [415] almost simultaneously ascertained a marked tumor-inhibiting effect from spleen tissue. The former observed, following implantation of spleen to animals, a refractory period against tumor inoculation, lasting about two weeks, the latter also obtained curative effects by means of spleen implantation, particularly when the organ was derived from animals previously treated with tumor tissue. Similar findings have been communicated by several authors; in particular, simultaneous injections of spleen and tumor tissue were found to delay or to cancel tumor formation.[416–423] It seems significant that an inhibition of Ehrlich mouse carcinoma could be achieved by simultaneous implantation of normal spleen and tumor, but not when the spleen employed originated from mice which had been treated with tar for fifteen to thirty days.[424] Somewhat divergent results were announced by another worker. Simultaneous implantation of Ehrlich mouse carcinomas and of spleen pulp of normal mice at first increased tumor growth, whereas spleen pulp of tumor-bearing mice delayed tumor growth; yet in later stages of the tumor process these differences disappeared.[425] Another approach of "spleen therapy" is represented by the ligation of the splenic vein, which measure led to regressions of Jensen rat sarcomas [426] or of Bashford mouse carcinomas [426a] and produced a marked immunity of animals against subsequent tumor inoculation.[427]

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[413] C. Bayer: Prag. med. Wchnschr. *35*: 1 (1910).

[414] W. H. Woglom: J. Exper. Med. *12*: 29 (1910).

[415] A. Braunstein: Berlin. klin. Wchnschr. *48*: 2029 (1911).

[416] C. Lewin & S. Meidner: Ztschr. f. Krebsforsch. *11*: 364 (1912).

[417] P. Biach & O. Weltmann: Wien. klin. Wchnschr. *26*: 1115 (1913).

[418] G. Domagk: Zentralbl. f. Path. *60*: 108; 125 (1934).

[419] P. Q. T. Tibiricá: Ann. Fac. Med. Sao Paolo *10*: 331 (1934); *11*: 43; 63 (1935); abstr. Ztschr. f. Krebsforsch. *43*: Ref. 239 (1936).

[420] F. M. Chaletzkaya: Arch. biol. Nauk *38*: 631 (1935); abstr. Ztschr. f. Krebsforsch. *44*: Ref. 56 (1936).

[421] F. P. Tinozzi: Ztschr. f. Krebsforsch. *36*: 372 (1932).

[422] V. Baroni & O. Comsia: Compt. rend. Soc. de biol. *111*: 1106 (1932).

[423] V. Baroni: 1st Internat. Cancer Congress, Madrid *1*: 453 (1933).

In further elaboration of this problem, spleen extracts have been used for increasing the resistance of mice against tumor transplantation [428, 429] or they were found to be effective in inhibiting tumor growth in animals.[430–432] Some of these extracts prepared from spleen and other reticulo-endothelial tissues were characterized by special qualities, inasmuch as they contained lipase-activators,[433] or proteolytic enzymes,[434] and favorable effects, resulting from administra-

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424. F. M. Chaletzkaya: Arch. biol. Nauk *40*: 101 (1936); abstr. Ztschr. f. Krebsforsch. *45*: Ref. *72* (1937).

425. D. Donati: Pathologica *31*: 249 (1939).

426. H. Auler: Ztschr. f. Krebsforsch. *30*: 295 (1928).

426a. Y. Nagumo: Gann *31*: 64 (1937); abstr. Am. J. Cancer *34*: 595 (1938).

427. W. Caspari & H. Flörcken: Ztschr. f. Krebsforsch. *36*: 546 (1932).

428. K. Yamagiwa: Tr. Jap. Path. Soc. *11*: 155 (1921).

429. K. Yamagiwa, S. Tsukuhara & S. Morimoto: Jap. Med. World *7*: 259 (1927); abstr. Cancer Rev. *3*: 167 (1928).

430. A. Braunstein: Ztschr. f. Krebsforsch. *32*: 118 (1930).

431. G. de Gaetani: Tumori n. s. *5*: 477 (1931).

432. E. Fodor, G. Erös & S. Kúnos: Ztschr. f. Krebsforsch. *38*: 1 (1932).

433. N. Waterman: Ztschr. f. Krebsforsch. *40*: 377 (1934).

434. V. Ghiron: Ztschr. f. Krebsforsch. *39*: 358 (1933).

tion of these preparations, accordingly, cannot be solely associated with the reticulo-endothelial function.

Special attention must be devoted to the work of some authors who, encouraged by the results of animal experimentation, introduced a reticulo-endothelial substitution therapy into the treatment of human neoplasia, although it will be shown that these treatments did not result in definite success. A systematic clinical treatment of neoplasia has been proposed by Theilhaber [435] in the form of implantation of spleen, bone marrow and thymus, derived from freshly slaughtered animals, and this therapy was believed to be effective particularly in the prevention of recurrences. However, convincing evidence for this claim has not been furnished, and no reports on further experiences with this treatment are available. Following extensive animal experimentation, Fichera [436] arrived at the conclusion that the reticulo-endothelial system has as its function the maintaining of an equilibrium in the body which prevents the formation of malignant tumors, and in elaboration of this view he developed a cancer therapy consisting of injections of a product, called I G 365. This preparation represented extracts of "antiblastic" organs, viz., of spleen, bone marrow, lymph nodes and thymus, and Fichera and his collaborators [436–438] reported that by careful selection of cases to be treated, cures, proven by histological examination, have been obtained in seven to eight per cent of tumor patients. However, the therapeutic results observed by other workers with the commercial preparation were far

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435. A. Theilhaber: Wien. klin. Wchnschr. *27*: 206 (1914); Wien. med. Wchnschr. *85*: 1394 (1935).

436. G. Fichera: Ztschr. f. Krebsforsch. *14*: 46 (1914); *36*: 1 (1932); *41*: 151 (1935); Strahlentherapie *50*: 302 (1934); loc. cit. 6.

437. G. de Gaetani: Arch. soc. ital. chir. 707 (1931); abstr. Ztschr. f. Krebsforsch. *35*: Ref. 36 (1932).

438. B. Capaldi: Radiol. Rundschau *3*: 10 (1934); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 167 (1934).

less favorable [439, 440] or entirely negative.[442–446] In evaluating these results, the possibility should be considered that the freshly prepared substances used by Fichera may have been superior to the commercial product.

Another preparation for cancer treatment, viz., splendothelan, has been elaborated and tested by Braunstein.[217, 447] This product consists of extracts of animal spleen and other reticulo-endothelial organs, supplemented by electrolytes, particularly by earth alkalis. Peroral administration of splendothelan was reported to cause growth inhibition of Jensen rat sarcomas and Ehrlich mouse carcinomas,[448] and Braunstein [449] described the following observations as support for the assumption that the product in question stimulated the reticulo-endothelial function. A marked increase of circulating monocytes occurred in treated animals; the blood of rats which had received splendothelan strongly inhibited the growth of neoplastic tissue cultures and, moreover, the preparation appeared to influence the metabolism, inasmuch as the erythrocytes of treated animals exhibited an increased oxygen consumption. The author found definite evidence that the splendothelan treatment was followed by beneficial effects in tumor patients, especially when used in combination with radiation therapy, or as a measure for increasing the general resistance of the

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439. Dieterich: abstr. Ztschr. f. Krebsforsch. *41*: Ref. 190 (1935).
440. D. Ho-ming: abstr. Ztschr. f. Krebsforsch. *45*: Ref. 154 (1937).
441. Klöppner: Schweiz. med. Wchnschr. *65*: 1033 (1935).
442. Baensch: München. med. Wchnschr. *81*: 1293 (1934).
443. T. Hünermann: Monatschr. f. Krebsbekämpf. *4*: 33 (1936); Ztschr. f. Hals-u. Ohrenheilk. *36*: 326 (1934).
444. G. Schulte & W. Lütteken: Strahlentherapie *52*: 247 (1935).
445. E. Domanig: Wien. klin. Wchnschr. *49*: 847 (1936).
446. F. Rusca: Helvet. med. acta *3*: 822 (1936).
447. A. Braunstein: 1st Internat. Cancer Congress, Madrid *1*: 744 (1933).
448. S. Konsuloff: Ztschr. f. Krebsforsch. *41*: 336 (1935).
449. A. Braunstein: Ztschr. f. Krebsforsch. *32*: 118 (1930); *39*: 321 (1933).

body, and for prevention of recurrences and the formation of metastases.[447, 450] Similar favorable experiences of the clinical use of splendothelan have been reported,[439, 451] but animal experiments performed by other workers only partly confirmed the antiblastic effect of splendothelan: Tar cancer of mice was not influenced by this preparation as to the yield of tumors and the formation of metastases, but the life of the animals was markedly prolonged.[452] Splendothelan feeding to mice of an inbred strain with a spontaneous tumor incidence of seventy per cent depressed the tumor frequency to forty per cent, but the growth of already developed neoplasms was not found inhibited by the treatment.[453] A preventive splendothelan treatment of mice was observed to increase the resistance of the animals against inoculation of Ehrlich sarcoma, a very virulent neoplasm, but in this instance, also, the preparation failed to inhibit existing tumors.[454] However, later investigations yielded no conclusive proof for either a prophylactic or a curative effect of splendothelan on animal tumors.[455, 456] Nevertheless, the aforementioned findings suggest that splendothelan may activate the reticulo-endothelial function, and consequently increase the resistance to neoplasia; thus it seems desirable to collect further experiences with this treatment, both in animal and clinical experiments.

Conceptions of a hormone function of the reticulo-endothelial system stood in the foreground of the work of Brüda

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450. A. Braunstein: 2nd Internat. Cancer Congress, Brussels *2*: 456 (1936).

451. A. Edinger: Med. Klin. *30*: 711 (1934).

452. N. Waterman: Acta Cancrol. *2*: 285 (1936).

453. N. Dobrovolskaia-Zavadskaia & P. Zéphiroff: Compt. rend. Soc. de biol. *121*: 1051 (1936).

454. A. Bessemans & L. Asaert: Ann. Inst. Pasteur *57*: 631 (1936).

455. G. Roskin & A. Maslowa: Bull. Biol. et Méd. exper. URSS *3*: 18 (1937).

456. A. Bessemans & E. von Thielen: Ztschr. f. Krebsforsch. *51*: 244 (1941).

and Pfeiffer.[402] These authors assumed, on the basis of parabiosis experiments, that the spleen secretes an antiblastic substance, called by them mesenchymin. Support for this assumption was also seen in the observation that neoplastic tissue cultures exhibited a considerably better growth when cultivated in the plasma of splenectomized animals and, furthermore, they reported a tumor-inhibiting action of a spleen extract (splenotrat) both in vivo and in vitro,[457] which latter finding has been confirmed by other workers.[458] For therapeutical purposes Brüda and Pfeiffer[459] recommended a method for obtaining the active endocrine factor of the spleen from sera of animals, the reticulo-endothelial system of which had been stimulated by colloid-storing or by "immunization." However, the results which may be expected of this treatment appear to be confined to prophylaxis, since in animal experiments mesenchymin rather prevented the development of transplanted tumors than inhibited their growth. More recently, it has been announced by other investigators[460, 461] that an "anticancer hormone" may be isolated from reticulo-endothelial organs, and favorable results with this preparation ("blasthormone") were claimed to occur in inoperable cancer patients. Yet, since the authors failed to give any details as to the preparation and the nature of this "hormone," and since no corroborating findings have been presented by other investigators, no evaluation of these results can at present be given. An interesting approach to supplement the depressed reticulo-endothelial activity in neoplasia by artificial means has been chosen by Kolisher, who in collaboration with Saphir and Horn[462] treated neoplasms by peritumoral injection of living macrophages; these cells had been segregated from spleens of animals, and the treatment mentioned yielded promising results when applied to rat tumors and also to some inoperable human cancers. According to the authors, continued work of this kind would require large amounts of macrophages, preferably cultivated in vitro from human macrophages, but so far no further reports on such experiments have been published.

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[457] B. E. Brüda: München. med. Wchnschr. *76*: 1671 (1929).

[458] F. Ludwig & J. von Ries: Schweiz. med. Wchnschr. *65*: 5 (1935).

[459] B. E. Brüda & H. Pfeiffer: Ztschr. f. d. ges. exper. Med. *68*: 554 (1929).

[460] A. Hirschfeld & G. Stark: Wien. med. Wchnschr. *87*: 405 (1937).

[461] A. Hirschfeld: Rev. franç. d'endocrinol. *15*: 392 (1937).

[462] G. Kolischer: Urol. & Cutan. Rev. *40*: 301 (1936).

A certain relation to the reticulo-endothelial system seems to be inherent in the combinative therapy proposed by Opitz and his school.[463-465] The preparation employed by these authors contained four factors, each of which was supposed to result in a special effect: trypan blue, for activation of the reticulo-endothelial function; adrenalin, for counteracting the internal secretion of the pancreas; choline, for stimulation of the parasympathicus, and cerium chloride, for an alteration of the mineral metabolism. The authors reported favorable effects from this treatment in animal experiments and also in some clinical cases. A partial identity with the reticulo-endothelial cells may also be assumed for the lymphoid cell system, the stimulation of which by fatty acids has been regarded as a means of tumor prophylaxis, on the basis of animal experiments.[466, 467]

In addition to the studies previously discussed, reference must be made to some work of more recent date which elaborated the problem under consideration. Thus, injections of spleen extracts have been found to cause the inhibition and even the regression of transplanted mouse carcinomas, and histological examinations proved these effects, on the one hand, to be expressed in a severe damage of the neoplastic

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463. E. Opitz: Ztschr. f. Krebsforsch. *22*: 108 (1925).
464. F. Kok & K. Vorlaender: Strahlentherapie *15*: 561 (1923).
465. K. Vorlaender: Klin. Wchnschr. *4*: 1120 (1925).
466. J. B. Murphy & J. J. Morton: J. Exper. Med. *22*: 800 (1915).
467. W. Nakahara: J. Exper. Med. *41*: 347 (1925).

tissue, and on the other hand, to be accompanied by a proliferation of reticulo-endothelial and mesenchymal tissue.[468] The active substances in spleen extracts have been more clearly analyzed by means of fractionation. Alcoholic spleen extracts were found to exert only a weak antiblastic effect; but after treating these extracts with benzene, the remaining benzene-insoluble fraction showed a far stronger tumor-inhibiting action, and accordingly it was believed that the benzene extraction had removed substances with a tumor-promoting quality which partly cancelled the tumor-inhibiting effect.[469]

Extensive investigations of the effect of spleen extracts on mouse tumors have been undertaken by Lewisohn and his collaborators.[470-472] The products were prepared by extraction of spleen with water, adsorption to activated carbon, triple extraction with alcohol, and elution; it was stressed that the resulting sterile aqueous solution was highly concentrated, inasmuch as one c.c. corresponded to 100 grams of fresh spleen tissue. Subcutaneous injections of 0.5 c.c. of this preparation were administered daily to mice seven to nine days after inoculation of sarcoma 180, and this treatment was continued for three to six weeks. The following effects were observed: hemorrhages occurred in the tumors after four or five days of treatment, and in sixty per cent (one hundred seventy out of two hundred eighty-one animals treated) complete disappearance of the tumors was observed

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468. K. Ulesco-Stroganowa: Akuš. i ginek. *1*: 27 (1936); abstr. Ztschr. f. Krebsforsch. *44*: Ref. 194 (1936).

469. E. Bauer, F. Chaletzkaja, A. Lyslowa & L. M. Schabad: Acta Cancrol. *2*: 17 (1936).

470. R. Lewisohn: Surg., Gynec. & Obst. *66*: 563 (1938); 3rd Internat. Cancer Congress, Atlantic City, 1939.

471. R. Lewisohn, R. Leuchtenberger & D. Laszlo: Surg., Gynec. & Obst. *71*: 274 (1940).

472. R. Lewisohn, C. Leuchtenberger, R. Leuchtenberger & D. Laszlo: Am. J. Path. *17*: 25 (1941).

after two to six weeks of treatment. In two hundred and ninety control animals receiving no treatment at all, or injections of other organ extracts, only eight per cent of tumor regressions took place, and the author emphasized the importance of administering large doses of spleen extract, since smaller doses (0.1 c.c. daily) even resulted in somewhat of a stimulation of the tumor growth. From this observation it was deduced that many of the previous investigations had led to negative results on account of insufficient concentrations and dosages of spleen extracts employed. Concerning the mechanism of the action of spleen extract, it appears very significant that in animals successfully treated, a considerable spleen enlargement—frequently four to five times the normal size—was found, a finding which was interpreted as indicating that the effective substances do not act directly on the tumor, but instead indirectly, by increasing the functional activity of the spleen. In continued work thirty per cent of cures were also accomplished by an analogous treatment of spontaneous mouse tumors,[471] and further details as to the preparation of the spleen extracts and their properties have been presented.[472]

This survey would be incomplete and one-sided unless opinions derived from experimental results were to be presented, opinions which appear to be in open contradiction to the theoretical assumptions and practical implications described in the foregoing. At first a great number of studies must be mentioned in which splenectomy or blockade of the reticulo-endothelial system with various colloids failed to result in a stimulation of tumor growth or, in some instances, even appeared to inhibit the neoplastic process.[473-483] How-

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473. N. Dobrovolskaia-Zavadskaia & N. Samssonow: Compt. rend. Soc. de biol. *92*: 1222 (1925).
474. W. Munck: Ztschr. f. Krebsforsch. *26*: 377 (1928).
475. J. G. Sanchez-Lucas: Ztschr. f. Krebsforsch. *28*: 374 (1929).

ever, according to the writers' opinion, the interpretation of these negative results should not necessarily imply that the aforementioned theories on the significance of the reticulo-endothelial function for neoplasia cannot be sustained. For certain experimental factors must be taken into consideration for a proper evaluation of the divergent findings. As to blockade with colloids, it must again be stressed that this interference does not regularly impair the reticulo-endothelial activity, but depending on the dosage employed, stimulation may even result.[cf 400] But also in those instances in which the blockade has brought about a definite damage of reticulo-endothelial elements, this effect may soon be concealed by a compensatory hypertrophic regeneration of other reticulo-endothelial tissue, and therefore attention must be given to the time factor. The latter consideration must be particularly emphasized as far as effects of splenectomy, i.e., of a quantitative reduction of reticulo-endothelial tissue is concerned. Another group of investigations arrived at negative findings as to the possibility of heterotransplantation to animals with an injured reticulo-endothelial function,[484–488]

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476. F. Bischoff, L. C. Maxwell & H. J. Ullmann: Proc. Am. Soc. Biol. Chem. 1931, p. 80.

477. A. Wagner: Hosp. tid. 1932. 817; abstr. Ztschr. f. Krebsforsch. *38*: Ref. 55 (1932).

478. H. Beck: Ztschr. f. Krebsforsch. *36*: 617 (1932).

479 E. Pribram: Ztschr. f. Krebsforsch. *39*: 399 (1933).

480. R. Segre: Cancro *4*: 236 (1933); abstr. Am. J. Cancer *23*: 128 (1935).

481. E. Tatarinov & R. Dinšiz: Ž. med. ciklu *3*: 367 (1933); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 106 (1935).

482. G. A. Kreuzwendedich von dem Borne & R. E. J. ten Seldam: Ztschr. f. Krebsforsch. *36*: 93 (1932).

483. C. F. Branch: Am. J. Cancer *26*: 110 (1936).

484. V. Scaffidi & R. Lanzillotta: Atti Acad. Lincei Roma *25*: II: 505 (1916).

485. L. Haendel & E. Haagen: Arch. f. Hyg. *103*: 298 (1930).

486. G. O. E. Lignac & G. A. Kreuzwendedich von dem Borne: Ztschr. f. Krebsforsch. *29*: 169 (1929).

487. D. Psaromitas: Ztschr. f. Krebsforsch. *30*: 77 (1929).

488. J. Heiman: Am. J. Cancer *23*: 282 (1925).

but the failure of producing tumor growth under these conditions can hardly be considered conclusive proof against the involvement of the reticulo-endothelial system in tumor pathology. A similar attitude seems justified regarding reports, according to which the injection of tumor filtrates, or action of other ordinarily noncarcinogenic stimuli (scarlet red, kieselgur) did not induce tumor formation in animals with damaged reticulo-endothelial systems [489] or in which blockade failed to influence the development of tar cancer.[490] One may be warranted in attributing a greater significance in this connection to the positive results obtained in similar experiments described above by assuming that certain, as yet unaccountable, factors were responsible for the failure of reproducing these findings regularly. On the other hand, objections have been raised against the active participation of the reticulo-endothelial function in tumor pathology, because the injection or feeding of spleen tissue or extracts was not seen to influence development and growth of Jensen rat sarcomas.[491] But here again it is doubtful whether the experimental conditions employed justified such general conclusions. In particular, these views have been countered by the statement that the Jensen sarcoma, as a tumor of mesenchymal origin, cannot be regarded as a proper object for studies concerned with the reticulo-endothelial function.[492] Although several previously mentioned investigations, leading to positive results, have also been undertaken with sarcomatous tumors, the writers wish to remark that some of their own experiences indicated that a different reactivity of the reticulo-endothelial system seems to prevail in hosts with tumors of epithelial, or of mesenchymal, origin, respectively. While the existence and significance of conflicting findings as to the involvement of the reticulo-endothelial system in neoplasia will by no means be denied, according to the writers' view. they do not represent a definite refutation of the theories, outlined previously, which regarded the reticulo-endothelial function as a decisive factor in the development and growth of malignant tumors.

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[489] W. Büngeler: Frankfurt. Ztschr. f. Path. *35*: 67 (1927).

[490] A. Satoh: Ztschr. f. Krebsforsch. *35*: 574 (1932).

[491] H. Druckrey: Klin. Wchnschr. *15*: 401 (1936); München. med. Wchnschr. *84*: 943 (1937); Ztschr. f. Krebsforsch. *47*: 112 (1938).

[492] H. Cramer: München. med. Wchnschr. *84*: 943 (1937).

Biological stimulation of reticulo-endothelial function. Attempts of this kind are based on a lesson taught by nature itself, since it is common knowledge, derived from phenomena of inflammation and of immunology, that in response to certain pathologic stimuli the body increases its natural resistance not only by mobilizing pre-formed reticulo-endothelial elements, but also by transforming "resting" mesenchyme into "active" reticulo-endothelial tissue. Correspondingly, a number of studies aiming at a stimulation of the reticulo-endothelial system in neoplasia tried to imitate these natural processes, and in this connection it will be recalled that, in fact, artificial production of bacterial infections, or administration of bacterial substances, have been employed in the treatment of neoplasia, as detailed in the part dealing with unspecific biological therapy. Indeed, reference has been made to theories of still broader implications, which envisaged the possibility of reducing the mechanisms of therapeutic effects achieved by widely different means, such as inorganic or organic compounds, "specific immunization," or unspecific biologic stimuli, to one common factor, namely, a functional activation of the mesenchyme and the reticulo-endothelial tissue.[493-495] In particular, Caspari [97, 493] defined this mesenchymal stimulation as being due to "necro-hormones," a term which he attached to tissue breakdown products, formed by

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493. W. Caspari: Strahlentherapie *18*: 17 (1924).
494. C. Lewin: Ztschr. f. Krebsforsch. *27*: 138 (1928).
495. F. Blumenthal & H. Auler: Ztschr. f. Krebsforsch. *35*: 283 (1932).

the action of a great variety of substances introduced into the body for therapeutic purposes. Actually, several investigations furnished histological evidence that beneficial effects derived from certain treatments are accompanied by a stimulation of the reticulo-endothelial system. Observations of this kind were reported in connection with growth inhibitions of mouse carcinomas, following intraperitoneal implantation of liver, spleen and testicle;[496] proliferations of mesenchymal tissue were found in mice treated with isamine blue ([497], cf. chapter II) and, as suggested in chapter I, the effects of lead treatment may depend on a similar mechanism.

In addition to the therapeutic measures named, the mechanism of which has been interpreted as being based on a reticulo-endothelial stimulation, several ways have been devised for deliberately accomplishing the same purpose. As mentioned above an "anti-reticular serum" has been produced by injection of bone marrow and liver into horses, and this serum was found to depress the reticulo-endothelial function of the treated animals.[412] Yet, as is almost regularly encountered in studies of the reticulo-endothelial function, the same stimulus may be employed for reducing or for increasing the reticulo-endothelial activity, according to the dosages employed. Actually, administration of small doses of the serum has been reported to represent an effective means of overcoming the mesenchymal anergy of tumor-bearing animals,[383] and this treatment resulted in cures of transplanted mouse tumors, and also in some beneficial effects when applied to tumor patients.[498, 499] Physical stimuli have also been found capable of increasing the reticulo-endothelial function. Among such procedures, reference is made to the ultraviolet irradiation of the spleen, made possible by the insertion of transparent windows into the abdomen of mice. This treatment has been reported to have caused cures of Ehrlich carcinomas in a great percentage of cases, while in other animals treated the tumor growth was strongly inhibited.[500] Similarly, total body irradiation of rabbits with ultraviolet rays was found to inhibit the growth of Kato rabbit sarcomas and the involvement of the reticulo-endothelial function in this effect was made probable by determinations of Congo Red storage, before and during the treatment.[501] A problem of much greater significance is represented by the effect of roentgen radiation on the reticulo-endothelial function. In this connection, it must be recalled that it has frequently been suggested, that the curative action of roentgen rays does not depend solely on a direct destruction of tumor cells, but also on an activation of the surrounding mesenchymal tissue.[493, 494, 502, 503] Such a view is supported by the experimental findings that a previous exposure of the skin of rats to roentgen radiation resulted in an immunity against subsequent tumor inoculation.[504] Although other workers failed to confirm this phenomenon,[505] a later study furnished additional corroborative evidence, inasmuch as the injection of roentgen-irradiated mouse skin was found to produce an absolute immunity against tumor transplantation in mice thus treated.[506] Fur-

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[496] U. Bertolotto: Sperimentale *85*: 331 (1931).

[497] Cramer; Wallbach: Klin. Wchnschr. *9*: 41 (1930).

[498] M. P. Fedjušin: Nov. chir. arch. *41*: 534 (1938); abstr. Ztschr. f. Krebsforsch. *51*: Ref. 255 (1941).

[499] A. A. Bogomolets: Acta med. URSS *2*: 65 (1939); abstr. Ztschr. f. Krebsforsch. *50*: Ref. 433 (1940); 3rd internat. Cancer Congress, Atlantic City, 1939.

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500. G. Roskin: Vestn. Rentgenol. *13*: 349 (1934); abstr. Ztschr. f. Krebsforsch. *42*: Ref. 80 (1935); Libro de Oro, Buenos Aires, p. 1204 (1935); Cancer, Bruxelles *11*: 91 (1934).

501. H. Kawaguchi: Jap. J. Obst. & Gynec. *23*: 178 (1940).

502. C. Lewin: Ztschr. f. Krebsforsch. *32*: 127 (1930).

503. A. Calò: Strahlentherapie *46*: 529 (1933).

504. J. B. Murphy, J. Maisin & E. Sturm: J. Exper. Med. *38*: 645 (1923).

505. A. Lacassagne & N. Samssonow: Compt. rend. Soc. de biol. *92*: 1224 (1925).

506. E. Urbach & A. Wiedmann: Wien. klin. Wchnschr. *45*: 862 (1932).

thermore, direct irradiation of the spleen of rats was reported to cause a marked growth inhibition of Flexner-Jobling carcinomas,[507] and the tumor formation in mice painted with methylcholanthrene was inhibited by weak roentgen doses, whereas high doses were found to stimulate the neoplastic process.[508] The latter study confirms the validity of a general concept,[509] that decisive importance must be assigned to the intensity of the stimulus which is applied to mesenchymal or reticulo-endothelial tissue. In the case of roentgen rays, small amounts may result in a stimulation, whereas a paralyzing effect may be caused by radiation amounts exceeding a certain threshold and thus causing irreversible cell damage. A similar view has also been expressed lately by an author on the basis of a review of the literature regarding the effects of total body irradiation in neoplasia. The results of previous work as well as of his own experiments led him to the conclusion that small radiation doses increase the resistance of the tumor host, and accordingly cause growth retardation, whereas large doses cause the opposite effects.[510]

In this connection it may be appropriate again to make reference to a striking phenomenon which has been encountered on several occasions in the course of this treatise, namely, the peculiar fact that the same chemical or physical agent (radio-active elements, arsenic, roentgen rays, carcinogenic hydrocarbons) has been discovered to possess two opposite qualities—the quality of inducing neoplasia, and the quality of counteracting neoplasia to a lesser or greater extent. While the possibility cannot be denied that special mechanisms may be involved in every one of these instances, in the writers' minds, this dual action may be hypothetically interpreted as depending on an interference with the reticulo-endothelial function, which, in the case of carcinogenesis, may be assumed to have been paralyzed by the stimulus in action, whereas antiblastic effects could be caused if the same stimulus were capable of increasing the reticulo-endothelial activity. It is, however, fully realized that particularly as far as human pathology is concerned, the mechanism involved is certainly not as simple as some animal experiments would indicate, for it is hardly possible to assume that merely the intensity of a stimulus is responsible for a resulting tumorigenic or an antiblastic effect. On the contrary, it must be expected that here the most decisive influence will be exerted by the reactivity of the reticulo-endothelial system, a quality which probably varies in each individual case—even changing during the lifetime of the individual—and is determined by a great many yet unknown factors.

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[507] H. Matsuoka: Jap. J. Obst. & Gynec. *17*: 25 (1934).

[508] E. Taschner, G. Gottlieb & M. Spritzer: Compt. rend Soc. de biol. *124*: 955 (1937).

[509] H. Chambers & G. Scott: Brit. J. Exper. Path *5*: 161 (1924/25).

[510] H. J. Bagg: Am. J. Roentgenol *40*: 418 (1938).

Chemico-pharmacological activation of the reticulo-endothelial system. It has been shown that the weakness of the reticulo-endothelial substitution therapy lies in the difficulty of preparing adequate extracts for such purposes and, moreover, it has in no way been proven that the cellular insufficiency of the reticulo-endothelial system can be successfully compensated by the administration of such extracts. On the other hand, the achievement of a biological activation of the reticulo-endothelial function has to cope with the problem of choosing the right quality and quantity of the stimulus to be employed, so as to avoid the danger of impairing, instead of increasing, the efficiency of the reticulo-endothelial system by these procedures. One way to solve these problems appears to lie in the creation of a pharmacology of the reticulo-endothelial system by studying and analyzing the influence of definite chemical substances on partial functions of these cellular structures. Experimental work has up to now furnished only the starting point for such an undertaking. Accordingly, endeavors to utilize measures of this kind for a treatment of neoplasia are still lacking in basic foundation, and are only of a preliminary nature.

The phenomenon that colloid-storing (blockade) of reticulo-endothelial cells only temporarily impairs their functions, and is followed by a compensatory hypertrophy, has been used in a few investigations with tumor-bearing animals, but since this effect is highly undependable, the results which have been achieved are not too encouraging. Small doses of colloidal silver solutions (collargol) have been administered to rabbits bearing sarcomas,[381] and Congo Red solutions were injected into mice inoculated with sarcoma 180. Although the latter treatment was not found to be very effective, its clinical use was recommended, less on account of the stimulating influence of Congo Red on the reticulo-endothelial system than on a basis of its hemostyptic quality, and also as a measure for combatting roentgen sickness in irradiated patients.[511]

More promising aspects seem to be exhibited by several recent findings, according to which an activating influence on the reticulo-endothelial system may be ascribed to a number of definite chemical substances of biological importance. As stated in chapter II, evidence has been furnished for such a quality of lecithin,[512] and attempts to employ this substance for therapeutical purposes have been discussed. A constituent of nucleic acids, viz, the pyrimidine base cytosine, has been found markedly to increase the colloid-storing ability of reticulo-endothelial cells, even when prior to the cytosine administration blockade of the cells had been performed with india ink.[513] These results have been confirmed, inasmuch as cytosine injections accelerated the resorption of injected bilirubin in animals after this function had been impaired by splenectomy,[514] and an activation of the reticulo-endothelial function, as shown by the results of Congo Red tests, has been observed in rabbits which received injections of uracil,[515] a substance chemically related to cytosine. Furthermore, it has been demonstrated that intravenous injections of colloidal carotene solutions stimulated the reticulo-endothelial function of rabbits,[516] and a similar effect has been independently obtained with vitamin A.[517] Since then several studies have furnished evidence for a close interrelationship of this vitamin with the reticulo-endothelial system. It has been shown that vitamin A is chiefly stored in the reticulo-endothelial elements of the liver, since the vitamin A storage was impaired only by interferences with the function of these cells, but not by injuries afflicted to the parenchymatous liver cells.[518, 519, 519a] The urinary vitamin A excretion, it has been suggested, depends on the reticulo-endothelial activity, since a parallelism was detected between this phenomenon and the impaired reticulo-endothelial function shown by the results of Congo Red tests.[520, 521] On the other hand, excessive vitamin A administration resulted in an increase of fat and lipid storage in the reticulo-endothelial elements of liver, spleen and bone marrow.[522] Lately, it has also been reported that a stimulation of the reticulo-endothelial function could

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[511] I. Arons & B. Sokoloff: Am. J. Roentgenol. *41*: 834 (1939).
[512] S. Amano & S. Hayashi: Gann *30*: 449 (1936).
[513] C. Moncorps & O. Günther: Klin. Wchnschr. *12*: 979 (1933).

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514. G. Ludány & L. Sarkady: Biochem. Ztschr. *294*: 101 (1937).
515. K. Stern: New Internat. Clinics s. IV, *3*: 111 (1941).
516. K. Stern & R. Willheim: Ztschr. f. d. ges. exper. Med. *97*: 354 (1935).
517. B. Ahmad: Current Sc. *2*: 477 (1934); abstr. Ronas Ber. *83*: 75 (1935).
518. F. Lasch: Klin. Wchnschr. *14*: 1070 (1935); *15*: 1036 (1937).
519. H. Wendt & D. König: Klin. Wchnschr. *16*: 1253 (1937).
519a: A. J. Cox: Proc. Soc. Exper. Biol. & Med. *47*: 333 (1941).
520. W. Thiele & K. Nemitz: Klin. Wchnschr. *18*: 95 (1939).
521. W. Thiele & U. Klodwig: Klin. Wchnschr. *18*: 821 (1939).
522. H. Noetzel: Ztschr. f. d. ges. exper. Med. *105*: 83 (1939).

be achieved in rats by means of nicotinic acid.[523] It may be recalled that a treatment of certain cancer cases with high doses of nicotinic acid yielded remarkable results,[524] as mentioned in chapter V. Several years ago experiments were started in which the influence of some of the substances named, viz., lecithin, carotene, uracil and cytosine, or mixtures of them, on the reticulo-endothelial functions of normal rabbits and on the Brown-Pearce rabbit carcinoma was studied by the writers. Although these investigations had to be discontinued prematurely, interesting results have been obtained which have been reported in preliminary form.[515] A resumption of this work on a broader scale is being considered in the hope that it may furnish controllable means of stimulating the reticulo-endothelial function, and thus combat the neoplastic process, at least insofar as control of growth for a certain period, prevention of recurrences and metastases, and support of surgical or radiation therapy are concerned.

Correlations of reticulo-endothelial functions to various phenomena connected with neoplasia. This concluding section is devoted to aspects considered by the writers to be very illuminating, namely, to an enumeration of a number of alterations of biochemical nature, apparent in neoplasia, which at the same time have been found to occur when the reticulo-endothelial function has been impaired in a tumor-free organism. These phenomena might therefore be interpreted as depending upon or being connected with this system and, thus, in addition to the evidence presented in the foregoing, new support could be derived from them for the assumption of a decisive participation of the reticulo-endothelial system in tumor pathology. However, the hypothetical nature of considerations of this kind is freely admitted, inasmuch as their unconditional acceptance would be in conflict with the laws of logic. For the fact that a phenomenon A is known to cause a phenomenon B does not justify the conclusion from observation of the phenomenon B that this has been caused by the phenomenon A in every special instance. Applied to the topic under discussion, this means that deviations from the normal which may be induced by impairment of the reticulo-endothelial system, when observed in neoplasia, need not necessarily be interpreted as being caused by a damage of the reticulo-endothelial system. Yet a cumulative incidence of such observations will at least increase the probability of a connection existing between the reticulo-endothelial function and miscellaneous alterations manifest in neoplasia, and may serve as a working hypothesis, the validity of which may then be tested by continued work; in the light of these reservations, the following survey may be accepted.

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[523] C. Scartozzi: Medicina Sperim., Arch. ital., Turin 6: 607 (1940).
[524] R. Willheim & F. Bocobo: Acta med. philipp. 2: 445 (1941).

Results of a certain interest in this connection concern inorganic biochemistry; a considerable lowering of the blood calcium concentration has been reported to occur following splenectomy.[525, 526] According to one study this calcium diminution was preceded by an initial calcium augmentation,[527] but one worker failed to confirm the change of the calcium concentration after splenectomy.[528] In this connection, changes of the blood calcium level, which were discussed in chapter I, are recalled, although they have not been proven to be of primary importance and regularity. The observation that a considerable potassium increase takes place in the bone marrow of cancer patients [529] could be interpreted as a chemical disturbance of a part of the reticulo-endothelial system, and a relation of this finding to the potassium accumulation

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525. H. A. Hall & E. Ablahadian: California & West. Med. *23*: 289 (1925).
526. E. B. Krumbhaar: Physiol. Rev. *6*: 160 (1926).
527. R. Paolini: Riforma med. *43*: 769 (1927).
528. F. P. Underhill & E. G. Gross: J. Biol. Chem. *81*: 163 (1929).
529. T. Hoffmann: Biochem. Ztschr. *243*: 145 (1931).

in neoplasms is conceivable. Certainly such an assumption is in need of continued investigation, as well as the claim that progressive tumor growth in rats is accompanied by a sodium decrease in the spleen of the animals whereas a sodium increase takes place in the tumors, blood and other organs. From these observations it has been inferred that the spleen of tumor hosts would secrete a sodium-containing factor of growth.[530]

A greater significance may be assigned to phenomena involving physicochemical aspects. First reference shall be made to the statement, derived by some authors from experimental work, that the reticulo-endothelial system exerts a regulating influence in general on the degree of dispersion of colloids in the body,[531] and this assumption may be regarded as being in agreement with the fact that one of the principal functions of reticulo-endothelial elements is the storing of particles exceeding a certain size. Actually it has been demonstrated that in neoplasia colloids of a more coarsely dispersed phase prevail in the humoral media (cf. chapter III). This could be considered as caused by an insufficiency of the colloid storing ability of reticulo-endothelial cells. But possibly a reciprocal influence exists, inasmuch as the colloidal state in the body in turn depresses the reticulo-endothelial activity, one worker having observed an impairment of the reticulo-endothelial system and a promotion of tumor growth following administration of globulin.[532] It may be thought that these proteins consisting of larger molecular aggregates block the function of reticulo-endothelial cells. The storage of colloids in reticulo-endothelial cells has been explained as

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530. D. C. A. Butts, T. E. Huff & E. C. Manz: Bol. inst. med. exper. para el estud. y. trat. d. cáncer *8*: 694 (1932).

531. S. Bratiano & A. Llombart: Compt. rend. Soc. de biol. *101*: 299 (1929).

532. J. R. Meyer: Compt. rend. Soc. de biol. *103*: 1322 (1930).

depending on differences between electrical potentials; a positive charge was assumed to be produced in the blood in circulation, especially in the capillaries, while colloids or microorganisms suspended in water were found to acquire negative potentials, and these differences in the electric charge were believed to attract these substances to the endothelial cells.[533] Alterations of electro-dynamic potentials have been reported to occur in tumor hosts (cf. chapter III) and it would be desirable to investigate whether these changes influence the storing of colloids in reticulo-endothelial cells. The surface tension appears to be connected with the reticulo-endothelial function, since it was found that surface-active substances are attracted to reticulo-endothelial cells.[534] Consequently, the impaired reticulo-endothelial activity in neoplasia may be held responsible for the accumulation of surface-active substances in the humoral and cellular media of tumor hosts and, in fact, the administration of spleen extracts was reported to cause a lowering of the surface activity of serum.[535] Only one publication is available as to a connection between the reticulo-endothelial system and hydrogen ion concentration. According to this study, blockade of the reticulo-endothelial system with collargol as well as splenectomy of animals raised the pH of the blood.[381] Further experimental analysis will be needed before it may be possible to consider the alkalosis, observed in certain types and stages of neoplasia, as a secondary phenomenon which is due to an impaired reticulo-endothelial function.

Striking resemblances are exhibited by the carbohydrate metabolism in neoplasia, on the one hand, and in insufficiency of the reticulo-endothelial system on the other. Splenectomy,

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533. F. Leiri: Acta med. Scandinav. *90*: 134 (1936).
534. Schulemann: Verhandl. d. pharmakol. Gesellsch., 10th meet.; Arch. f. exper. Path. u. Pharmakol. *157*: 75 (1930).
535. E. Bauer: München. med. Wchnschr. *72*: 1723 (1925).

or blockade, of reticulo-endothelial cells, has been found to result in a rise of the blood sugar values in fasting individuals, and in a lowering of the glucose tolerance.[536-540] It appears significant that parabiosis experiments made it probable that this influence of the spleen on carbohydrate metabolism is due to a hormone factor.[541] Blockade of the reticulo-endothelial system has been reported to impede the re-synthesis of lactic acid to sugar,[542] whereas administration of spleen extracts promoted glycogen formation in the liver.[543] Also increased proteid sugar values have been found in the blood of animals after blockade of their reticulo-endothelial system with india ink,[544] and thus, by means of inflicting damages to the reticulo-endothelial system, a number of alterations could be reproduced which have been encountered as secondary phenomena in tumor hosts. Yet, while this might be regarded as mere coincidence, the problem of tumor metabolism is much more intrinsically involved by taking into consideration the observation that deviations from the normal, characteristic for the vitro metabolism of neoplastic tissue, can be reverted to normal conditions by the addition of spleen extracts. The glycolysis is lowered, the respiration increased to such an extent that the original proportion between glycolysis and respiration of five to one is changed to one to two.[545] Hence, a function regulating metabolism may be ascribed to the reticulo-endothelial tissue and to the spleen

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536. T. Togawa: Biochem. Ztschr. *109*: 1 (1920).
537. F. Venulet: Ztschr. f. d. ges. exper. Med. *63*: 720 (1928).
538. A. V. Marx: Klin. Wchnschr. *9*: 2058 (1930).
539. E. S. London & J. B. Entin: Ztschr. f. physiol. Chem. *225*: 279 (1934).
540. A. Noma (1925); quot. K. Fuziwara: Biochem. Ztschr. *256*: 384 (1932).
541. E. Lauda: Wien. klin. Wchnschr. *46*: 481 (1933).
542. E. O. Gaessler: Ztschr. f. d. ges. exper. Med. *69*: 105 (1929).
543. K. Fuziwara: Biochem. Ztschr. *265*: 76 (1933).
544. Y. Matsuoka: J. Biochem. *24*: 355 (1936).
545. W. Büngeler: Frankfurt. Ztschr. f. Path. *43*: 409 (1932).

and an impaired activity of these organs in neoplasia may be responsible for the metabolic disturbances apparent in this malady. Actually, the spleen of tumor-bearing rats has been reported to exhibit a very high anaerobic glycolysis.[546] It may even be assumed that the reticulo-endothelial system participates in general in oxidative processes, since splenectomy, or blockade, has been found to impede the oxidative purine metabolism.[547] Furthermore, animals the reticulo-endothelial function of which had been damaged by blockade, have been reported as failing to respond in the same manner as normal animals—with an increase of hemoglobin and of glutathione—to a lack of oxygen induced experimentally.[548] In this manner certain information is available for a possible connection between the characteristic alteration of oxidation processes in neoplasia and the reticulo-endothelial function.

An involvement of the reticulo-endothelial system in the metabolism of lipids has been demonstrated by various experimental findings. Alimentary fat ingestion by blocked animals led to blood lipid curves, deviating from the normal values; [549] splenectomy, or blockade, was seen to result in a hypocholesterinemia,[549, 550] while administration of a spleen extract caused hypercholesterinemia,[551] and a regulation of the total sterol metabolism, exerted by the spleen, has been thought very probable.[552] It must suffice at present to refer to disturbances of the lipid metabolism described in chapter II as occurring in malignancy, whereas a definite correlation of these findings with an insufficiency of the reticulo-endo-

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546. Y. Okamoto: Proc. Imp. Acad. (Tokyo) *3*: 184 (1927); abstr. Ztschr. f. Krebsforsch. *25*: Ref. 73 (1927).

547. F. Chrometzka & H. Kühl: Ztschr. f. d. ges. exper. Med. *95*: 140 (1935).

548. G. Cronheim: Klin. Wchnschr. *12*: 1217 (1933).

549. S. Leites: Biochem. Ztschr. *186*: 391; 436 (1927); *198*: 157 (1928).

550. F. Goebel & H. Gnoinski: Biochem. Ztschr. *185*: 414 (1927).

551. E. Schliephake: Klin. Wchnschr. *12*: 1936 (1933).

552. C. A. Pellerano: Libro de Oro, Buenos Aires, p. 1848 (1935).

thelial system will be possible only on the basis of continued experimental work aimed in this direction. On the other hand, some evidence has been furnished of an influence of sterols and phospholipids on the storing of colloids by reticulo-endothelial cells; both in vivo and in vitro the cholesterol-lecithin ratio has been found to determine the extent of phagocytosis since the injection, or addition, of small cholesterol quantities, increased phagocytosis; whereas large cholesterol amounts showed the opposite effect, and lecithin had an influence inverse to that of cholesterol.[553] Hence it may be assumed that changes of sterol and phospholipid concentrations which have been found to take place in tumor pathology will, in turn, affect the partial function of reticulo-endothelial elements represented by phagocytosis.

The interdependence of reticulo-endothelial activity and of certain vitamins, particularly vitamin A and its pro-vitamin carotene, has been discussed previously. In addition, it may be mentioned that the reticulo-endothelial system has been thought to be involved in the development of spontaneous tumors in animals fed a diet characterized by a disturbed vitamin balance (poor in vitamin A, rich in vitamin B).[554] Evidence for this assumption has been furnished by histological examinations which showed in these animals a damage of reticulo-endothelial structures.[385] It must, however, be conceded that the experimental material pertaining to the influence of nutritional factors on the reticulo-endothelial system is as yet far too little to permit any definite statement. Likewise it must be deemed desirable to proceed with investigations which may analyze the relationship of the reticulo-endothelial system with the processes of normal growth, since work of this kind may throw some light on the

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553. H. Horster & E. Dorbath: Deutsches Arch. f. klin. Med. *178*: 289 (1938).
554. Rh. Erdmann & E. Haagen: Ztschr. f. Krebsforsch. *26*: 333 (1928).

differences existing between normal and neoplastic growth.

A number of interesting aspects have been revealed by studies devoted to the interaction of endocrine glands and the reticulo-endothelial function. Most of this work concerned the pituitary gland, and clear indications for a correlation of this organ with reticulo-endothelial activity have been made available. By means of histological methods a factor stimulating the spleen function was demonstrated as present in anterior pituitary extracts,[555] and an increase of the weight of the pituitary of twenty-five per cent was found in splenectomized rats. Histologically these animals exhibited a hypertrophy of the pituitary reticulo-endothelial elements.[556] Moreover, it was reported that splenectomy of humans or animals resulted in an increased excretion of urinary gonadotropic substances, and hence it was supposed that the normal spleen function restrains the excretion of gonadotropic factors and that the increased concentrations of urinary gonadotropins observed in some tumor hosts may be due to an impaired activity of the spleen.[557] On the other hand, factors have been isolated from the pituitary which appeared to influence the reticulo-endothelial activity. Histological examinations showed an increased storage of carmine in reticulo-endothelial cells, following the administration of corticotropic hormone;[558] another worker, employing the Congo Red test, ascribed a stimulating effect on the reticulo-endothelial function to a posterior pituitary extract and to gonadotropic factors, whereas the anterior pituitary growth hormone was found to depress the reticulo-endothelial activity. Total hypophysectomy, or administration of weak roentgen doses to the gland, resulted in an activation of the reticulo-endothelial system. Significantly, the effect of the factors mentioned on the tumors showed a depression of the reticulo-endothelial system to be accompanied by an increase of the tumor growth and, conversely, a stimulation of the reticulo-endothelial system to be associated with an impeded growth of the tumors.[559] Precise data as to pituitary factors influencing the colloid storing function of the reticulo-endothelial system have been furnished by extensive work performed during the last years.[560] Alkaline extracts have been prepared by special methods from the anterior pituitary, and their injections into rabbits were followed by a considerable increase of the reticulo-endothelial activity, indicated by Congo Red tests. The active principle ("positive restropic factor") was described as thermolabile, free of protein and not identical with any of the pituitary hormones known. By means of special extraction methods, the presence of this restropic factor in the blood of normal rabbits, horses and humans has been demonstrated, but it could not be detected in the blood of tumor patients. Extracts obtained from these persons either did not influence the colloid storing function at all, or lowered it, which latter phenomenon was attributed to the presence of a "negative restropic factor."[561] Experiments on animals yielded analogous results, inasmuch as the blood of normal rats was found to contain the positive restropic factor. But inoculation of Walker sarcoma to the animals was followed by the disappearance of this factor and, in later stages of tumor growth, by the appearance of the negative restropic factor.[562] Likewise, rabbits which normally showed the presence of positive restropic factors in their blood lost this property after treatment with carcinogenic hydrocarbons or after prolonged administration of roentgen radiation.[384, 385] Two significant facts must be stressed, which have been observed in these studies: one, these changes occurred prior to the morphological appearance of malignant growth, and two, they failed to take place when the animals were injected with noncarcinogenic hydrocarbons closely related chemically to the carcinogens employed.[384] Hence, a direct relationship between a carcinogenic mechanism and the impairment of the reticulo-endothelial activity seems to have been established, and an extension of similar investigations to other forms of tumor development may broaden the basis of this concept.

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[555] D. Perla: J. Exper. Med. *63*: 599 (1936).
[556] L. F. Edwards & C. W. Wright: Endocrinology *21*: 808 (1937).
[557] F. Sauerbruch & E. Knake: Klin. Wchnschr. *16*: 1268 (1937).
[558] M. Riess & I. Gothe: Endokrinologie *19*: 148 (1937).

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[559] K. Narimatsu: Jap. J. Obst. & Gynec. *20*: 407 (1937).

[560] C. Wetzler-Ligeti & B. P. Wiesner: Nature, London *140*: 892 (1937); *141*: 1100 (1938); Endocrinology *22*: 693 (1938).

[561] C. Wetzler-Ligeti & B. P. Wiesner: Brit. M. J. *2*: 444 (1938).

[562] T. Csato, C. Wetzler-Ligeti & B. P. Wiesner: Am. J. Cancer *36*: 262 (1939).

In the writers' opinion, future work may assign a far-reaching significance for tumor pathology to another trend of work in endocrinology; by this, reference is made to interesting studies in which blockade of the reticulo-endothelial system has been found to prevent the formation of antihormones, normally resulting from prolonged administration of gonadotropic factors.[563, 564] An extension of these observations has been accomplished by a communication of recent date; the internal secretion of ovarian tissue implanted into normal rats was found impeded by the action of some antihormone factors, but previous splenectomy or injection of trypan blue prevented this restraining effect; that is, the secretion of the ovarian implant proceeded unimpeded in animals the reticulo-endothelial function of which had been damaged.[565] Now it is extremely tempting to correlate these observations with certain phenomena discussed in connection with endocrine aspects of tumor pathology. As amply discussed in chapter

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[563] A. S. Gordon: Cold Spring Harbor Symposium, Vol. *5*: 419 (1937).

[564] A. S. Gordon, W. Kleinberg & A. H. Charipper: Proc. Soc. Exper. Biol. & Med. *36*: 484 (1937); Science *86*: 62 (1937); J. Exper. Med. *70*: 333 (1939).

[565] A. J. Miller & A. K. Lampton: J. Urol. *45*: 863 (1941).

VII, estrogens were found to induce tumor formation under certain experimental conditions, and this effect has been interpreted, on the basis of convincing evidence, as being dependent upon a disturbance of the hormone equilibrium in the body. In other words, excessive administration of estrogens apparently shifted the hormone balance in a direction conducive to tumor development; in the light of the experimental results quoted above, it may be assumed that such an endocrine alteration would presuppose an insufficiency of protective forces which tend to maintain the normal status, and experiments performed with splenectomy, or blockade, of animals seem to point to the reticulo-endothelial system as the carrier of such a function. There is no doubt that for the time being the trend of thoughts just expressed is essentially hypothetical, but the phenomena described appear of sufficient interest to warrant further work along these lines, involving comparative determinations of the reticulo-endothelial function in animals susceptible to tumor formation by means of estrogen administration and in those resistant to this stimulus.

The attention of the writers has been called to an involvement of the reticulo-endothelial system in tumor pathology in connection with the phenomenon of carcinolysis, which will be discussed in detail in chapter X. Some time ago, several authors demonstrated that extracts of reticulo-endothelial organs possessed a considerable carcinolytic activity,[436, 566, 567] and splenectomy of humans or dogs has been found to be followed by a marked diminution of the carcinolytic activity in the serum.[568] Furthermore, it appeared significant that spleen extracts of normal persons, but not of cancer patients, exhibited carcinolytic properties,[568] and that animals painted with tar showed a gradual diminution of carcinolytic factors

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566. N. Waterman: Biochem. Ztschr. *188*: 65 (1927).

567. N. Waterman, L. de Kromme & J. F. Lemmens: Biochem. Ztschr. *205*: 1 (1929).

568. G. C. Peracchia: Ztschr. f. Krebsforsch. *26*: 42 (1927).

in the serum.[569] On the other hand, administration of reticulo-endothelial extracts, especially of spleen extract, resulted in a definite increase of the carcinolytic activity of the serum,[570] and weak roentgen irradiation of the spleen had a similar effect, whereas high roentgen doses lowered the carcinolysis.[571] The latter effect may be understood easily in the light of previously mentioned results, according to which weak roentgen doses stimulate, and strong ones paralyze, the reticulo-endothelial activity. Incidentally, the proposal derived from these findings has been to utilize such stimulating spleen irradiations for therapeutical purposes.[572] As will be amplified in chapter X, it has been recognized that the characteristic quality of the sera of cancer patients is represented not so much by the loss of the carcinolytic property, but mainly by the appearance of factors which protect cancer cells in vitro against lytic factors.[573] For the purpose of studying the latter quality of serum in relation with the reticulo-endothelial function, studies have been performed in which normal rabbits were subjected to a blockade of the reticulo-endothelial system by means of india ink, trypan blue or carmine, and these interferences resulted in a change of the serum behavior, inasmuch as it resembled the serum of tumor hosts, i.e., exhibited a "cancer-cell-protecting" quality; similar results have been obtained by splenectomy of rabbits.[574] These findings have been accepted as warranting the conclusion that the appearance of "cancer-cell-protecting factors" can be considered as the expression of a reticulo-endothelial insufficiency. In other words, it may be assumed

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569. F. Piccaluga: Tumori *11*: 291 (1925).
570. M. Ferro: Tumori n. s. *6*: 1 (1932).
571. H. Zacherl: Klin. Wchnschr. *9*: 289 (1930).
572. F. von der Hütten: Strahlentherapie *13*: 197 (1922).
573. R. Willheim & K. Stern: Wien. klin. Wchnschr. *43*: 227 (1930); *44*: 728 (1931).
574. R. Willheim & K. Stern: Ztschr. f. Krebsforsch. *38*: 501 (1933).

that cancer-cell-protecting factors, whenever and wherever formed, would be rendered innocuous by a normally functioning reticulo-endothelial system, and that this system represents a barrier which must be broken down before these substances can permanently appear in the body. While these results derived from the carcinolytic phenomenon tested in vitro appeared mainly of theoretical interest, they acquired a heightened significance for tumor pathology in general after it could be demonstrated that cell suspensions of Ehrlich mouse carcinoma exhibited a considerable increase of virulence when they had adsorbed cancer cell protecting factors prior to inoculation.[575] This result is in agreement with an earlier report, according to which treatment of rat sarcoma suspensions with serum of cancer patients augmented the tumor yield and accelerated the growth rate of the transplanted tumors.[576] Thus, at least one possibility has been clarified by these experiments, by which the impaired function of the reticulo-endothelial system may decisively influence the tumor process; that is, by permitting the formation and action of substances promoting tumor growth in the body. It must be left to a continued elaboration of these problems whether additional links in the chain of phenomena may be detected which connect alterations of the reticulo-endothelial system with various factors contributing to the development and growth of malignant tumors.

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575. K. Stern & R. Willheim: Ztschr. f. Krebsforsch. *39*: 54 (1933).

CHAPTER IX

BIOCHEMICAL ASPECTS OF TUMOR ORIGIN AND TUMOR GROWTH

IN ADDITION to the well defined biochemical approaches to the problem of cancer which have been extensively studied and have formed the subject of the preceding chapters, it appears necessary to survey phenomena which investigations of the last years demonstrated to be of undeniable importance for tumor pathology, but which, for the time being, cannot be classed with any of the previously discussed angles of biochemical cancer research. First, hereditary factors have clearly been recognized as determining the incidence and localization of certain malignant tumors, and this will be discussed in the first part of this chapter, as far as biochemical and experimental research has contributed to the elucidation of this subject. The second part will deal with factors which have been investigated regarding their ability to stimulate or restrain normal or neoplastic growth, a trend of work which will be shown to attempt a clarification of problems of both theoretical and practical significance. Finally, a separate discussion must be reserved for certain biochemical and experimental results pertinent to malignant tumors, the cause of which was established as being an infection with viruses of a specific nature.

1. *Heredity and Extra-Chromosomal Factors.*

Attention has been frequently directed by clinicians to a cumulative incidence of neoplasia in certain families, and numerous reports have been published in support of the assumption that hereditary factors are, in part, responsible for the development of malignant tumors. Since a detailed account of the statistical data collected on this subject cannot be given here, reference is made to other treatises,[1-4] but it is noteworthy that, according to authoritative opinion,[cf 1] only a subordinate role was assigned to heredity as far as human malignancy is concerned. For, even admitting that genetic factors determine the constitution of an individual, and thus his susceptibility to various tumorigenic stimuli, it is—on the other hand—easily comprehensible that unaccountable factors of environment may modify the reactivity of the organism. Moreover, the establishment of a definite correlation between incidence of human neoplasia and genetic factors appears hardly feasible in view of the complexity of transmission of hereditary qualities in man.

It was to be expected that experiments on animals would offer more precisely controllable conditions to permit a much clearer evaluation. In fact, extensive work of this kind has been continued for several decades by a number of investigators who contributed greatly to the clarification of this problem and recorded the spontaneous tumor incidence in inbred animal strains.[5-9] While here again readers interested in particulars of this work are referred to the original articles

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[1] J. Ewing: Neoplastic diseases, W. B. Saunders, Philadelphia & London 1940, pp. 94–100.

[2] R. J. Behan: Cancer, C. V. Mosby, St. Louis 1938, pp. 28-36.

[3] H. R. Schinz & F. Buschke: Krebs und Vererbung, G. Thieme, Leipzig 1935.

[4] M. T. Macklin: Familial incidence of cancer; in "A symposium on Cancer," The University of Wisconsin Press, Madison 1938, p. 32

[5] M. Slye: Ztschr. f. Krebsforsch. *13*: 500 (1913); J. Med. Res. *30*: 281 (1914); *32*: 153 (1915); J. Cancer Res. *1*: 479 (1916); *2*: 401 (1917); *4*: 207 (1919); *5*: 25 (1921); *11*: 335 (1927); Am. J. Path. *17*: 655 (1941).

[6] C. J. Lynch: J. Exper. Med. *42*: 829 (1925).

[7] L. C. Strong: J. Cancer Res. *10*: 219 (1926).

[8] C. C. Little: Am. J. Cancer *15*: 2780 (1931).

[9] R. Korteweg: Nederl. tijdschr. f. geneesk. *77*: 4034 (1934).

and to reviews,[10, 11] the fundamental results of one exhaustive series of studies on the subject has led to the following conclusions. The susceptibility to tumor development is most probably inherited by genes of recessive mendelian type, and these genetic factors affect specific organs, whereas the resistance against neoplasia appears to be linked with a dominant mendelian gene.[5] Although many other investigations essentially corroborated these statements, some opposing views have been expressed on the basis of experimental evidence. A discussion of these divergent opinions is not attempted, this being outside of the scope of this book, yet it may be anticipated that convincing evidence was furnished for the fact that hereditary transmission of tumor susceptibility is, in addition to genetic factors, also dependent on extra-chromosomal factors and that influences of environment in post-uterine life can decisively modify the resistance, or susceptibility, of animals.

Since this outline is concerned mainly with problems of biochemical cancer research, emphasis is laid on the following achievements derived from studies on hereditary tumor development in inbred animal strains. One, by breeding strains exhibiting a high incidence of spontaneous tumors, oncologists have been provided with a subject suitable for investigations of varied cancer problems, since a closer resemblance may be assumed to exist between these spontaneous animal tumors and human neoplasms than is the case in transplanted tumors. Two, the precise knowledge, made available as to spontaneous tumor frequency in certain inbred animal strains,

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10. L. Kreyberg: The Genetic and Constitutional Aspects of Spontaneous and Induced Tumors; in "A symposium on cancer," University of Wisconsin, Madison 1938, p. 3.

11. C. C. Little: The Influence of Intrinsic Factors on the Development of Tumors in Mice; in "A symposium on cancer," University of Wisconsin, Madison 1938, p. 20; J. Nat. Cancer Inst. *1*: 727 (1941).

12. F. R. Selbie: Brit. J. Exper. Path. *17*: 348 (1936).

makes possible a more accurate evaluation of the action of carcinogenic stimuli when compared with similar experiments using animals of unknown genetic qualities. The neglect of these factors obviously reduced to a marked degree the value of some older studies in which carcinogenic effects were observed in a comparatively small percentage of animals. This gives rise to doubts as to whether this tumor development had not been spontaneous, rather than due to the carcinogen employed. Three, it may be expected that a deeper theoretical understanding of tumor pathology can be obtained from studies performed with animals of known genetic qualities in the event that it might be possible to change their susceptibility, or resistance, to neoplasia by post-uterine interferences.

A problem of great importance has frequently been studied in connection with genetic factors, namely, the susceptibility of animals to transplanted tumors. In this regard, as stated in chapter VIII, great variations have been observed and more recent work demonstrated the possibility of breeding strains exhibiting characteristic qualities. Thus, rat strains have been obtained, which were highly susceptible to the inoculation of Jensen sarcoma,[12] and successful transplanting of a spontaneous mouse sarcoma to mice of different strains was found to depend on genetic factors inherent in the strain to which the individual animal belonged. A partial correlation of these genetic factors to hemagglutinogens present in the respective strains was made probable.[13] Very recently, the literature dealing with the influence of hereditary factors on susceptibility to inoculated tumors was reviewed and new evidence furnished demonstrating the importance of these factors for the transplanting of tumors to rats.[14] Likewise, tumors which were induced in mice of a pure strain by means of dibenzanthracene, were found to be transplantable only to mice belonging to the same strain, whereas inoculation into mice of other genetic characteristics resulted in regression after an initial growth.[15] On the other hand, interesting observations were reported as to an apparently hereditary transmission of resistance to transplanted tumors. When rats, in which Jensen sarcomas had receded spontaneously or were brought to regression by roentgen radiation, were mated with each other, the offspring of these animals, when inoculated with Jensen sarcoma, showed a markedly higher percentage of tumor regressions than control animals (forty per cent as compared with ten per cent), and the size of persisting tumors reached, on the average, only half the size of tumors in controls.[16]

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[13] P. A. Gorer: J. Path. & Bact. *44*: 691 (1937); *47*: 231 (1938).

[14] J. L. Orbison, H. A. Davenport, F. B. Aneen, D. D. Spicer & R. M. Galt: Cancer Research *1*: 891 (1941).

A large amount of work has been devoted to the question as to whether animals of strains with high incidence of spontaneous tumors exhibit a reactivity toward carcinogens, distinguishing them from animals of different genetic character. Actually, it has been possible to breed mouse strains which showed an increased susceptibility to the carcinogenic action of coal tar,[17] or of methylcholanthrene,[18, 19] but it was stressed that the breeding of animals resistant to cancer was not possible.[18] Likewise, an acceleration of the tumor development, due to the administration of 9, 10 -dimethyl-1, 2-benzanthracene, was observed in a particular inbred mouse strain, when compared with the reaction of animals of other lineage.[20] On the other hand, the spontaneous tumor frequency in inbred strains may be enhanced by carcinogenic stimuli, since tar treatment was found to increase the total incidence, or the number of tumor nodules, respectively, in mice with a hereditary tendency to malignant pulmonary tumors.[21] Similarly, administration of methylcholanthrene stimulated the formation of mammary cancer in susceptible mice.[22] However, less uniform results have been obtained in another study performed with mice of a high-mammary-cancer strain. As compared with control animals, dibenzanthracene injections reduced the frequency of breast cancer by 5.9 per cent, whereas treatment with radon tubes resulted in an increase of 5.2 per cent. It was believed that the tumor development in these cases was primarily due to endogenous factors, and not to the exogenous carcinogens, but final conclusions as to the interaction of intrinsic and extrinsic stimuli could not be drawn from these observations.[23] This leads to a number of investigations which attempted to clarify this problem by comparing the development of chemically induced neoplasms in pure animal strains with different genetic qualities. In general, it has been concluded from such experiments that the action of extrinsic carcinogens is limited by intrinsic hereditary factors; that is, certain stimuli were assumed to induce neoplasia only in susceptible animals, whereas they fail to do so in refractory animals. The susceptibility was regarded as inherited and specifically to affect certain organs.[24] However, as will be shown in the following, experimental evidence of continued work in this direction has only partly corroborated this concept. Mice belonging to strains of different hereditary constitutions, as shown by the incidence of spontaneous tumors, were exposed to carcinogens, such as dibenzanthracene, or infection with parasites, and the observation was made that in animals of a strain with a low cancer incidence, local development of mostly sarcomatous neoplasms occurred in response to the carcinogens but the frequency of spontaneous tumors was not increased.[25] Injections of dibenzanthracene or of methylcholanthrene were given to mice of two different pure strains, one of which exhibited a high, and the other a low mammary tumor incidence. Animals of the former strain were found more susceptible and animals of the latter, more resistant to carcinogenesis.[26] However, in continuing this work, the susceptibility of highly inbred mouse strains to spontaneous tumors, to transplanted tumors, and to the action of carcinogens was compared, and the results obtained were interpreted to indicate that no definite correlation between these various forms of neoplasia could be established. That is, no strain has been found which proved resistant, or susceptible, to all types of tumor growths named.[27] These results may explain the divergent findings derived by different workers from studies on this problem. Painting with tar of mice bearing spontaneous cancer did not yield any evidence that development of tar cancer was influenced by the existing neoplasms, or vice versa,[28] and only slight differences were noted as to the development of tumors following the administration of dibenzanthracene in mice belonging to high, or low, tumor strains. It was assumed that the genetic resistance of the animals could be either overpowered by the extrinsic stimulus or was confined to spontaneous tumor formation.[29] On the other hand, another investigator reported that tar painted mice of a high mammary cancer strain showed a somewhat decreased yield of tumors when compared with low tumor strains. He stated: "This constitutes further evidence that the carcinogenic response of the skin to a carcinogenic agent, although in itself a strictly localized process, is nevertheless conditioned by systemic factors, lying outside the area subjected to the agent. As a result of this relationship the simultaneous occurrence of skin cancer and mammary cancer is rare, and the incidence of skin cancer in tarred mice diminishes as the actual incidence of spontaneous mammary cancer increases. This may account for the statistical phenomenon observed in man that different populations may show an approximately equal total incidence of cancer, with a widely different organ incidence." [30] The latter statement touches upon a highly significant attempt to explain varying distributions of neoplastic localizations in human pathology, and will be expanded later. As far as experiments on animals are concerned, corraborating evidence for this assumption was furnished by some studies. Painting with dibenzanthracene produced tumors in mice of a low tumor strain almost twice as frequently as in animals of a high tumor strain,[31] and a high resistance against the carcinogenic action of benzpyrene was observed in mice of a high mammary cancer strain.[32] Yet, just the opposite findings were obtained not only in some of the older studies mentioned but also in an investigation of recent date. When female mice of a strain with a spontaneous incidence of mammary cancer of sixty-three per cent were painted with methylcholanthrene,

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[15] M. R. Lewis: Bull. Johns Hopkins Hosp. *67*: 325 (1940).

[16] S. Russ & G. M. Scott: Proc. Roy. Soc., London s. B *128*: 126 (1939).

[17] L. Kreyberg: Acta path. et microbiol. Scandinav. *11*: 174 (1934).

[18] G. M. Bonser: J. Path. & Bact. *46*: 581 (1938).

[19] G. B. Mider & J. J. Morton: Am. J. Path. *15*: 299 (1939).

[20] J. Engelbreth-Holm & H. Lefèvre: Cancer Research *1*: 102 (1941).

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[21] C. J. Lynch: Proc. Soc. Exper. Biol. & Med. *43*: 186 (1940).

[22] J. Engelbreth-Holm: Cancer Research *1*: 109 (1941).

[23] N. Dobrovolskaia-Zavadskaia & N. Adamova: Bull. Assoc. franç. p. l'étude du cancer *28*: 76 (1939).

[24] C. J. Lynch: Am. J. Clin. Path. *6*: 293 (1936).

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[25] N. Dobrovolskaia-Zavadskaia & N. Adamova: Bull. Assoc. franc. p. l'étude du cancer *27*: 308 (1938).

[26] H. B. Andervont: Pub. Health Rep. *53*: 229 (1938).

[27] H. B. Andervont: Pub. Health Rep. *53*: 1647 (1938).

[28] G. M. Bonser & K. I. Connal: J. Path. & Bact. *48*: 263 (1939).

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[29] N. Dobrovolskaia-Zavadskaia: Compt. rend. Soc. de biol. *121*: 1268 (1936).

[30] W. Cramer: J. Path. & Bact. *43*: 77 (1936).

[31] C. F. Branch: Am. J. Cancer *26*: 110 (1936).

[32] C. Dittmar: Ztschr. f. Krebsforsch. *50*: 20 (1940).

the presence of a tumor in one organ was not seen to prevent the tumor development in another, but rather to facilitate it. In mice, which eventually developed mammary cancer, warts, due to the methylcholanthrene painting, were seen earlier, and conversely, the appearance of mammary cancer was noted earlier in mice painted with methylcholanthrene than in unpainted animals.[33] The apparent sharp contrast exhibited in the results of these studies certainly represents an incentive to continued work in this line. The divergencies may have been due to some differences in the genetic qualities of the animals tested and, in support of this view, a study may be mentioned in which hybrids of different inbred mouse strains were used to ascertain the susceptibility to spontaneous, and to induced, tumors. It was found that the susceptibility to both types of tumors is linked with dominant genes involving multiple genetic factors and, hence, the susceptibility of hybrids to various types of tumors cannot be predicted.[34] Furthermore, the different results of the chemical induction of tumors in animals of inbred strains may have been caused by factors of environment not accounted for and, in this respect, the following will show to what extent extrinsic interferences may modify hereditary susceptibility, or resistance, to neoplasia.

There have been frequent occasions in preceding chapters to enumerate a variety of exogenous influences which were found to modify the resistance, or susceptibility, to neoplasia. Whereas the precise interpretation of most of these experiments is rendered difficult because endogenous factors, i.e., the hereditary constitution of the animals employed was undetermined, it is interesting to point to studies performed with inbred animal strains. In this regard dietary factors were ascertained to be capable of modifying the hereditary quali-

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33. G. M. Bonser & L. M. Wainman: J. Path. & Bact. *52*: 263 (1941).
34. H. B. Andervont: J. Nat. Cancer Inst. *1*: 135 (1940).

ties. Thus, feeding of wintergreen (gaultheria) oil to mice of a high tumor strain was followed by an appreciable reduction in number and delay in the appearance of spontaneous cancers ([35], cf. chapter II). Also, the development of tar cancer in mice of a pure strain exhibited certain variations according to the dietary regime employed. In particular, feeding of fresh liver enhanced the formation of warts but not the rate of their malignant transformation, and it increased the total yield of tumors. Feeding of thyroid substance somewhat accelerated the malignant transformation and increased the total number of tumors. These disclosures were regarded as proof that hereditary reaction to carcinogens may be partially transformed by dietary measures, in this case, probably by lowering the genetically determined resistance of some of the animals.[36]

Special attention must be devoted to the influence of pregnancy on the incidence of mammary cancer in mice of inbred strains, a phenomenon apparently connected with endocrine factors and outlined in chapter VII. Extensive studies were performed showing that the incidence of breast cancer is considerably increased in female mice of pure strains when the animals are permitted to breed rapidly. The tumor yield in these animals exceeded by far that of virgin mice, and the condition of increased breeding activity was considered as representing a "functional test," which permits one to ascertain the genetic qualities of animals more precisely as to their susceptibility to spontaneous tumor development.[37, 38] In a later study it was found that hybrid female mice—obtained by mating animals of a strain susceptible to tumors with those of a tumor-resistant strain—showed a higher cancer incidence

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35. L. C. Strong: Am. J. M. Sc. *192*: 546 (1936).
36. L. Kreyberg: Acta path. et microbiol. Scandinav. Suppl. *37*: 317 (1938).
37. H. J. Bagg: Am. J. Cancer *27*: 543 (1936).
38. H. J. Bagg & J. Jacksen: Am. J. Cancer *30*: 539 (1937).

when exposed to repeated pregnancies.[39] However, other workers who studied the influence of breeding on tumor incidence, arrived at somewhat different interpretations. That is, by reviewing the literature and on the basis of their own experiments, it was concluded that the final frequency of tumors was the same in non-breeding as in breeding animals, and that repeated pregnancies merely accelerated the tumor development.[40, 41] Since endocrine factors in the form of estrogens were recognized to be of great importance in the development and growth of certain types of neoplasia (cf. chapter VII), comparison of the sexual function of animals belonging to pure strains having a different tumor susceptibility was suggested. Some differences of the estrous cycles were found to exist in mice of high, or low, tumor strains, but the establishment of a correlation between the sexual function and cancer frequency was not possible,[42, 43] and a later study on this problem arrived at no definite conclusion, since an apparent relationship between the occurrence of diestrus and of tumor incidence might have been due to the longer survival of the control animals free of tumors.[44] Likewise, continued investigations will be required for evaluation of disturbances of the estrous cycles observed in rats of a pure strain exhibiting a high mammary tumor incidence and a low fertility.[45] The promoting influence of estrogens on the development and growth of neoplasms of the female

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39. W. U. Gardner: Cancer Research *1*: 345 (1941).
40. H. B. Andervont & W. J. McEleney: Pub. Health Rep. *53*: 777 (1938).
41. R. Bierich & A. Rosenbohm: Ztschr. f. Krebsforsch. *50*: 387 (1940).
42. E. L. Burns, M. Moskop, V. Suntzeff & L. Loeb: Am. J. Cancer *26*: 56 (1936).
43. V. Suntzeff, E. L. Burns, M. Moskop & L. Loeb: Am. J. Cancer *26*: 761 (1936).
44. L. C. Strong: Am. J. Cancer *32*: 80 (1938).
45. J. M. Wolfe, E. Burack & A. W. Wright: Am. J. Cancer *38*: 383 (1940).

sex organs and of the breast is extensively discussed in chapter VII, as well as the tumorigenic effect of estrogens on male animals of strains with high mammary cancer incidence in females. It may be supplemented that, lately, analogous experiments have shown that estrogen administration to males of a high cancer strain increased the tumor incidence in the animals in proportion to the hormone quantities employed.[46] Estrogen treatment of one year's duration was used in female mice of a hybrid strain,[cf. 89] and it resulted in a frequent appearance of lesions, some of them malignant, of the uterine cervix. In this connection it was stressed that no relationship exists between genetic factors, responsible for the development of mammary cancer, and the factors determining the incidence of uterine tumors.[47] Similar views of a more general nature have been expanded by other authors, inasmuch as it was stated that non-epithelial tumors in mice obeyed different genetic laws than mammary cancer; mendelian laws were discernible only in the latter instances, whereas factors of an endocrine nature—involving a disturbance of the hormone equilibrium—may be important for the development of non-epithelial tumors.[48]

An extremely interesting phenomenon has been detected, representing a transmission of susceptibility to neoplasia, which is definitely not associated with genetic factors, although it must be regarded as an hereditary influence. This refers to the discovery that the susceptibility of newborn mice of a pure strain, exhibiting a high mammary cancer frequency, can be decisively changed when they are nursed, not by their own mothers or other animals of the same strain, but by mice of a strain with different genetic qualities as to tumor incidence. Bittner,[49] who first reported on such experiments, observed that foster-nursing of mice of a high cancer strain by mothers of a low tumor strain reduced the frequency of spontaneous mammary cancer in the foster-nursed animals to twenty per cent, whereas eighty per cent occurred in controls nursed by mice of the high tumor strain. This effect was described as a hereditary influence, since it is determined by a factor present in the milk of animals of the parent strain, but as being of extra-chromosomal nature, i.e., not depending on genetic factors which obviously cannot be changed by fostering. The author substantiated these findings and obtained new evidence that the milk of animals, belonging to a high mammary cancer strain, contained a factor responsible for the development of tumors in the offspring, since exclusion of this "milk-influence" by means of fostering by animals of a low tumor strain resulted in a diminished tumor incidence of these animals.[50] On the basis of further experiments it was concluded that the incidence of spontaneous breast cancer in mice is due to the combined action of three factors. One, to the breast cancer producing influence transmitted to the litter by the milk of mothers of a susceptible strain; two, to an inherited susceptibility of genetic nature; three, to a stimulating influence of certain hormones. The first factor alone was not found to be sufficient to induce neoplasia, since offspring of a low tumor strain fostered by mothers of a high tumor strain did not result in an increased tumor incidence among these animals.[51] It was especially stressed that for the purpose of effectively reducing the tumor incidence in the offspring of a high cancer strain, the fostering by animals of a low cancer strain must set in very early, namely, within four hours after the birth. A rise of the cancer incidence in a litter of a low tumor strain was seen when the animals were nursed by high cancer strain mothers, but only when the fostered young animals possessed the required genetic qualities.[52] These observations were corroborated by the work of several authors.[48, 53, 54] In one study the fostering of a low-tumor-strain litter by high-tumor-strain mothers produced a definite increase of breast incidence in the fostered animals,[55] which apparently possessed a latent genetic susceptibility. Furthermore, it was found that fostering of high-tumor-strain mice by low-tumor-strain mothers reduced the susceptibility of the fostered animals to the induction of mammary cancer by means of estrogens, and the opposite effect resulted from fostering of low-tumor-strain offspring by high-tumor-strain mice,[56] a finding which corroborates the concept of Bittner.[51] No final explanation can so far be given for the mechanism of this extra-chromosomal "milk-influence." It was stressed that the hereditary transmission of this factor is not governed by mendelian laws,[57, 58] and an analysis of these factors has been presented.[59] The observation that an "active milk-influence" may arise in a low cancer strain, although not exhibiting an increased mammary cancer incidence, has been interpreted to speak for the presence of a virus, or a similar agent, in the "active milk."[60, 60a] This hypothetical factor was shown as present outside of milk, since the transplantation of normal tissue (thymus, spleen, lactating breast) of high cancer mice to low cancer animals had the same effect of increasing the susceptibility to cancer as foster-nursing [61] and, later, analogous results were obtained by feeding low cancer strain offspring with an extract prepared from frozen spontaneous mammary cancer.[62] Likewise, recent experiments demonstrated a "mammary-cancer-promoting" factor present in the blood and tissues of high-cancer-strain animals. The term "mammary tumor inciter (MTI)" was proposed for the active factor.[63] This agent was also shown to be present in fresh saline extracts of spontaneous mammary carcinomas of mice; feeding of these extracts induced mammary tumors in susceptible mice.[63a] Ultracentrifugation of the milk of mice with positive MTI-influence was found to sediment the greatest part of the active factor; [63a] other workers reported complete sedimentation of the MTI-factor after centrifugation of the mouse milk.[63b] These findings were believed to support the assumption of a virus nature of the factor under discussion.

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[46] E. L. Burns & J. R. Schenken: Proc. Soc. Exper. Biol. & Med. *43*: 608 (1940).

[47] E. Allen & W. U. Gardner: Cancer Research *1*: 359 (1941).

[48] C. C. Little, W. S. Murray & A. M. Cloudman: Am. J. Cancer *36*: 431 (1939).

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[49] J. J. Bittner: Am. J. Clin. Path. *7*: 430 (1937); Am. J. Cancer *30*: 527 (1937).

[50] J. J. Bittner: Am. J. Cancer *35*: 90 (1939).

[51] J. J. Bittner: Am. J. Cancer *39*: 104 (1940); Proc. Soc. Exper. Biol. & Med. *45*: 805 (1940).

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[52] J. J. Bittner: J. Nat. Cancer Inst. *1*: 155 (1940).

[53] K. B. De Ome: Am. J. Cancer *40*: 231 (1940).

[54] W. A. Barnes & R. K. Cole: Cancer Research *1*: 99 (1941).

[55] H. B. Andervont: J. Nat. Cancer Inst. *1*: 147 (1940).

[56] M. B. Shimkin & H. B. Andervont: J. Nat. Cancer Inst. *1*: 599 (1941).

[57] W. S. Murray & C. C. Little: Am. J. Cancer *27*: 516 (1936).

[58] J. J. Bittner: Cancer Research *1*: 793 (1941).

[59] W. S. Murray: Cancer Research *1*: 123 (1941).

[60] J. J. Bittner: Cancer Research *1*: 113 (1941).

[60a] C. H. Andrewes: Proc. Roy. Soc. Med. *33*: 75 (1939).

It is comprehensible that attempts have been made to connect the genetic susceptibility of certain inbred animal strains to tumor development with other biochemical and physiological qualities, by which means a distinction might be made between tumor-susceptible and tumor-resistant animals. Inconclusive results, which were derived from studies of the sexual cycle, have been mentioned previously, but other approaches appeared more successful. Thus, trichloracetic acid precipitates of blood of mice belonging to different inbred strains were studied and the changes in the properties of these precipitates, which occurred during the aging of the animals, were believed to be a criterion for the hereditary tumor susceptibility of the animals.[64, 65] Furthermore, a lowering of the hemoglobin values was found to occur in cancer-susceptible mice to a more marked degree, and at an earlier age, than in cancer-resistant animals, and this was thought to represent an indication of premature aging.[66, 67] In connection with this observation, male mice belonging to strains with a high or low incidence of mammary cancer in females of the same strain were tested as to their tolerance against the "antioxydant" salicylic aldehyde. The tolerance was found to depend on weight, age, hemoglobin level, and genetic qualities of the animals and, accordingly, the tolerance was lower in animals with low hemoglobin values and was inversely proportional to the frequency of spontaneous mammary cancer in the females of the same strain. Since cancer resistant mice were found more resistant to the toxic effect of salicylic aldehyde than cancer susceptible animals, this reaction was declared to be a possibility for measuring the intrinsic cancer susceptibility, and it was believed that the resistance to the aldehyde depended on a physiologic oxidation-reduction mechanism which is important for tumor development.[68] Another factor of the genetically determined tumor susceptibility was assumed to be associated with the concentration of sulfhydryl groups in the body, differences of the respective values having been found by sulfhydryl determinations in newborn rabbits of different strains.[69] As will be described

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[61] J. J. Bittner: Pub. Health Rep. *54*: 1827 (1939).

[62] J. J. Bittner: Science *93*: 527 (1941).

[63] G. W. Wooley, L. W. Law & C. C. Little: Cancer Research *1*: 955 (1941).

[63a] W. Ray Bryan, H. Kahler, M. B. Shimkin & H. B. Andervont: J. Nat. Cancer Inst. *2*: 451 (1942).

[63b] M. B. Visscher, R. G. Green & J. J. Bittner: Proc. Soc. Exper. Biol. & Med. *49*: 94 (1942).

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[64] L. C. Strong & T. H. Werner: Am. J. Cancer *26*: 767 (1936).

[65] L. C. Strong: Am. J. Cancer *27*: 115; 118 (1936).

[66] L. C. Strong: Am. J. Cancer *27*: 500 (1936).

[67] L. C. Strong & L. D. Framis: Arch. Path. *23*: 202 (1937); Am. J. Cancer *38*: 399 (1940).

[68] L. C. Strong: Yale J. Biol. & Med. *12*: 255 (1940); Arch. Path. *32*: 420 (1941).

[69] F. S. Hammett: Science *83*: 57 (1936).

in the next part of this chapter, sulfhydryl groups were reported to exert characteristic influences on processes of cell growth and differentiation, and the participation of sulfhydryl compounds in tumor development has been detailed in chapters II and V. Finally, it may be recalled that biophysical measurements revealed characteristic differences between animals of tumor susceptible and tumor resistant strains, inasmuch as alterations of the electrodynamic fields of the body were found in the former, prior to the appearance of tumors.[70, 71]

Although the aforementioned discoveries are very interesting, in spite of their fragmentary nature, another trend of work is conceivable, which would attack the problem in question by directly investigating the chromosomal elements of the cell as the bearer of genetic transmission. Only preliminary work dealing with this extremely difficult undertaking has been reported so far, and the pertinent experiments were carried out with low animals. Thus, it was pointed out that irradiation, tar, and sex hormones, all of which constitute potential carcinogenic stimuli, have the quality in common of evoking chromosomal changes in drosophila, and it was hypothetically assumed that the mechanism of exogen carcinogenesis may involve similar chromosomal mutations,[72] a concept closely related to the older theory of cancer origin according to Boveri.[73] Very interesting results have been obtained lately by roentgen irradiation of gametes of sea urchins and frogs. When these irradiated gametes copulated, severe disturbances of the chromosomal apparatus expressed in abnormal development of the fertilized eggs (multipolar

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[70] H. S. Burr, G. M. Smith & L. C. Strong: Am. J. Cancer *32*: 240 (1938).

[71] H. S. Burr: Yale J. Biol. & Med. *13*: 783 (1941).

[72] B. M. Slizyński: Nature, London *137*: 536 (1936).

[73] Th. Boveri: The Origin of Malignant Tumors, Williams & Wilkins, Baltimore 1929.

divisions) and of the embryos became apparent.[74] Yet at the present time, no definite conclusions can be drawn from such experiments as to the primary involvement of chromosomal mutations in the general etiology of neoplasia, and as to the inheritance of such alterations being responsible for the transmission of cancer susceptibility.

As mentioned in the introduction to this survey, the element of heredity, and of genetic factors, represents an extremely difficult problem, so far as human neoplasia is concerned. While the extensive statistical material collected and published on the incidence of malignant tumors in various countries and among different human races cannot be included herein, it may be mentioned briefly that interesting observations were repeatedly made of variations in the localization of cancer in certain organs. That is, whereas the total incidence of neoplastic diseases may be the same, very marked differences were found in populations living in different geographic climates or environments or belonging to different racial groups, relative to the frequency, with which special organs are affected with malignant tumors.[cf. 75-77] Of course, it is almost impossible to ascertain to what degree these deviations are due to heredity, or due to factors of environment, since the different racial progeny is most frequently associated with differing food, clothing and other habits. On the basis of extensive statistical analyses an inverse proportion was found existing between the frequency of cancer of the skin and of internal organs. That is, populations with high skin

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74. P. S. Henshaw: J. Nat. Cancer Inst. *1*: 277 (1940).

75. R. Reding: Le terrain cancereux and cancérisable, Masson, Paris 1932.

76. F. L. Hoffman: The mortality from cancer throughout the world, Prudential Press, Newark 1915; Cancer and Diet, Williams and Wilkins, Baltimore 1937.

77. S. Peller: Ztschr. f. Krebsforsch *34*: 128 (1931); Acta Cancrol. *1*: 1 (1934).

cancer frequency exhibited a rarer incidence of internal tumors than populations with low skin cancer incidence, and it was also found that only a very small percentage of persons who had been cured of skin, or lip, cancer, later developed neoplasms of internal organs.[78, 79] The increased incidence of skin cancer was found to be in direct proportion to the exposure to sunlight, a phenomenon discussed in chapter III. Although some authors, on the basis of other statistical material, contested the validity of the concept envisaging an antagonism between incidence of skin cancer and of internal tumors,[80, 81] the author of the former studies maintained his views and furnished additional evidence in favor of his theory.[82] A corroborating study has been published and it was even stated that the frequent development of skin cancers in populations exposed to strong solar radiation produced a true immunity against formation of internal tumors.[83] No definite correlation to these findings can be assigned as yet to a recent report, according to which a high frequency of gastric cancer in Japan is accompanied by a very low incidence of skin cancer.[84] Possibly a number of factors, mainly of dietary nature, account for this deviation in the distribution of neoplasia. Another striking phenomenon in this category is commonly known, as the fact that carcinoma of the penis is extremely rare in circumcized males (Jews, Mohammedans) as compared with uncircumcized population groups. Less well known statistical evidence shows a lower incidence of cancer of the uterine cervix in Jewish women,

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78. S. Peller: Lancet *2*: 552 (1936); Am. J. Hyg. *29*: 34 (1939).
79. S. Peller & C. S. Stephenson: Am. J. M. Sc. *194*: 325 (1937).
80. K. C. Conrad & A. B. Hill: Am. J. Cancer *36*: 83 (1939).
81. S. Warren & O. Gates: J. A. M. A. *115*: 1707 (1940).
82. S. Peller: Am. J. Hyg. *34*: 1 (1941).
83. F. L. Apperly: Cancer Research *1*: 191 (1941).
84. M. Nagayo & R. Kinosita: Yale J. Biol. & Med. *12*: 301 (1940).

as compared with non-Jewish women.[85-87] It is conceivable that this might be due to the absence of a cancer-producing stimulus exerted by the smegma which accumulates in the preputium. Recently performed experiments failed to demonstrate a carcinogenic quality of smegma in mice,[88] yet such negative results, as is generally the case, are far less conclusive than positive ones, and continued work will be required for clarification of this problem.

2. *Growth-promoting and Growth-inhibiting Factors.*

In the course of the preceding presentations of various oncologic trends of work there has been frequent opportunity to point to the anomalous character of growth of malignant tumors as one of their most impressive properties. On the one hand, it had to be stressed that a great number of biochemical data which deviated from the normal in neoplasia cannot be regarded as being entirely specific for malignant tumors, since similar relations are exhibited by other rapidly growing tissues. On the other hand, there are doubtless indications present which suggest that neoplasms are distinguished from normal organs not only by the quantitative factor of an increased growth rate, but also by qualitative alterations of their growth pattern. Morphologically, this is expressed in the invasive growth of malignant tumors, which destroys and replaces neighboring tissues, in the tendency to form metastases, and in certain imponderable features which led pathologists to speak of the "autonomous," "lawless" or "egotistic" character of neoplastic growth. The great num-

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[85] M. Sorsby: Cancer and Race: The incidence of cancer among Jews, Bale, London 1937.

[86] P. Weir & C. C. Little: J. Hered. *25*: 277 (1934).

[87] I. Davidsohn: Med. Leaves, Vol. 2: 19 (1939).

[88] M. Fishman, M. J. Shear, H. F. Friedman & H. L. Stewart: J. Nat. Cancer Inst. *2*: 361 (1942).

ber of studies in which attempts have been made to characterize neoplastic growth more clearly, or to establish definite factors as responsible for the distinction of tumor growth from normal growth, may be classified into three groups. The first approached the problem directly by histological examinations of tumor cells and their mitotic activity, or by quantitative analyses of tumor growth in vivo or in tissue cultures; a second derived valuable information from an indirect attack of the problem, i.e., by investigating the effects of growth influencing factors isolated from malignant tumors; finally, a third group of studies will be surveyed in which a variety of agents were tested as to their properties of impeding, or enhancing the growth of neoplastic, or of normal tissues.

No detailed account can be given here of the first group of studies mentioned, because of their predominantly morphological character, and the reader is referred to pathology text books [cf. 89] for obtaining precise information, particularly as to alterations of nuclear structure and of mitotic mechanisms in tumor cells. A very interesting, but mainly hypothetical, explanation has been developed by Boveri [78] who envisaged the transformation of a normal into a malignant cell as being due to multipolar divisions leading to a change of the chromatin-complex of the nucleus. In consequence of this alteration, he assumed that either a chromosomal factor is lost which normally inhibits excessive mitosis and cell proliferation, or a new factor is added which stimulates these processes. While these hypothetical assumptions have so far not led to conclusive experimental elaboration, a great deal of work has employed the method of tissue cultures for an analysis of neoplastic growth. In this connection special attention is called to the studies of the influence of toxic substances on cell division and growth in vitro and in vivo, per-

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89. J. Ewing: loc. cit. 1, pp. 81–94.

formed by Dustin and his collaborators (summary, cf. [90]). These authors designated sodium cacodylate, colchicine and other substances tested by them, as "caryoclastic poisons," and their action on a great variety of plant and animal cells revealed interesting phenomena which the authors believed to be significant for the understanding of cancerous growth, inasmuch as the substances named attacked and selectively damaged dividing cells. Similar experiments with urethane, sodium cacodylate, auramine and colchicine have been carried out by Ludford [91] who observed the effect of these substances on tissue cultures of Crocker mouse sarcoma and of normal mouse tissues. It was confirmed that these toxic agents destroyed cells in the process of mitosis, while "resting" cells did not suffer irreparable damage, but the sensitivity of malignant cells to these substances was not found differing from that of dividing embryonic cells. Recently experiments have been reported in which the growth of tumor tissue cultures derived from surgical human material was studied for twenty-four hours, and their behavior was compared with that of benign tumor cultures. In general, the growth activity, as expressed in migration of cells and liquefaction of the medium, was found proportional to the malignancy, although some exceptions to this rule were observed, and the proposal was offered to use studies of twenty-four hour tissue cultures as an additional method for the determination of the grade of malignancy of neoplasms.[92] However, it does not seem permissible to reduce the characteristic behavior of neoplasms simply to the factors of intensity and velocity of growth, as has been proposed by an author who, in a theoretical discourse, rejected the assumption, that "lawlessness of growth" characterizes malignant tumors as "romanticism."[93] Evidence to the contrary, that is, disclaiming a characteristic behavior of the growth rate at least of transplanted animal tumors, has been furnished by a comparative analysis of Walker rat tumors and of rat embryos. Both tissues were found to exhibit in vitro and in vivo growth curves of corresponding order and course, and it was believed that the tumors are characterized, not by an abnormal proliferative quality, but by an impaired tendency to cell differentiation.[94] In support of this view, reference was made to analogous results obtained in older investigations with the Bashford mouse carcinoma,[95] with the Flexner-Jobling rat carcinoma[96] and with neoplastic tissue cultures.[97, 98] Doubtless a great obstacle for the clarification of the problem of neoplastic growth qualities is represented by the insufficient knowledge available as to the physiology and biochemistry of normal growth. For this reason great importance may be assigned to systematic efforts which have been undertaken along these lines and which were summarized recently.[99] Using marine hydroids, the influences were investigated which are exerted by biological constituents, such as amino acids, nucleic acids, and their components, on growth and development. The effects of these substances were determined separately as to cell differentiation, proliferation, regeneration, organization, and metaplasia. Emphasis may be placed on the findings that a promoting influence was observed on differentiation of the following compounds: d-glutamic acid, l-aspartic acid, l-proline, l-hydroxyproline, tyrosine, cytosine, and thymine. It was pointed out that cytosine, thymine and some of the amino acids mentioned show a common characteristic in the form of a pyrrolidone structure (CO-NH-CH), and in view of the fact that malignant cells are deficient as to their ability of differentiation, it is understandable that a closer investigation of these substances has been recommended in connection with the tumor process.[99, 100] Furthermore, attention has been called to a participation of sulfhydryl compounds in growth processes, since inunction of such substances (cysteine, cystine-d-sulfoxide, thiocresol, methionine, etc.) into the skin of mice was followed by cell proliferation, and differentiation and organization were markedly increased. Here, again, a connection with neoplasia was assumed to exist, inasmuch as qualitative and quantitative differences in the chemical constitution might be responsible for the deviating growth pattern of malignant tumors.[101] Additional evidence of the importance of sulfhydryl compounds for growth processes has been discussed in chapters II and V. Another basis for a chemical identification of substances with active growth influence was derived from studies on artificial induction of the embryonic development of amphibia. In this work, compounds of sterol nature were recognized as being effective, and the terms "organizer" and "evocator" were given to them. In view of the chemical relationship of carcinogenic hydrocarbons and of estrogens to sterols, it was thought possible that carcinogenesis might involve a mechanism similar to that observed in the experimental interference with embryonic growth and differentia-

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[90] A. P. Dustin: Arch. f. exper. Zellforsch. *22*: 395 (1939); 3rd Internat. Cancer Congress, Atlantic City 1939.

[91] R. J. Ludford: Arch. f. exper. Zellforsch. *18*: 411 (1936).

[92] E. J. Grace: N. Y. State J. Med. *41*: 459 (1941).

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[93] H. Oertel: Am. J. M. Sc. *197*: 1 (1939).

[94] R. Schrek: Am. J. Path. *12*: 525 (1936).

[95] E. F. Bashford: Rep. Imp. Cancer Res. Fund *4*: 131 (1911).

[96] K. Sugiura & S. R. Benedict: J. Cancer Res. *5*: 373 (1920).

[97] A. Carrel & A. H. Ebeling: J. Exper. Med. *48*: 285 (1928).

[98] A. Fischer, H. Laser & H. Meyer: Ztschr. f. Krebsforsch. *32*: 99 (1930).

[99] F. S. Hammett & S. P. Reimann: Ann. Int. Med. *13*: 1483 (1940).

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100. F. S. Hammett: Protoplasma *30*: 465 (1938).

100a. S. P. Reimann: Cause and Growth of Cancer, University of Pennsylvania Press, Philadelphia 1941; p. 29.

101. S. P. Reimann & G. Toennies: Arch. Path. *29*: 175 (1940).

tion.[102, 103] However, objections have been raised against the physiological significance of the theory on "organizers" of normal growth, since the effects of sterols were demonstrated as unspecific,[104, 105] and in general an application of data obtained with lower animals to cell physiology and pathology of mammals may be attempted only with great caution.

A controversial issue is raised by the important problem of the presence of growth promoting substances in neoplasms. A priori, it seemed conceivable that the increased growth rate of malignant tumors might be reflected in, or depend upon, the presence of such substances, and the term of "blastines" has been proposed for these hypothetical growth factors.[106] Particularly, it was thought possible that these substances, produced by the tumors themselves, may facilitate the formation of metastases, since histological examinations gave evidence that microscopic metastases are frequently present prior to the manifestation of macroscopic ones. Hence, it was assumed that the tumor cells first deposited are destroyed, and that substances with growth promoting properties are liberated from them, which, so to speak, prepare the soil for the development of macroscopic metastases.[107-109] A similar conclusion may be drawn from the observation in animal experiments that smaller quantities of tumor substance are required for transplantation when whole tumor pulp is used than when isolated tumor cells, freed from detritus and de-

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[102] J. Needham: Brit. M. J. *2*: 701 (1936); Proc. Roy. Soc. Med. *29*: 1577 (1936).

[103] C. H. Waddington, J. Needham, W. W. Nowinski & R. Lemberg: Proc. Roy. Soc., London s. B *117*: 289 (1935); *120*: 198 (1936).

[104] L. G. Barth & S. Graff: Cold Spring Harbor Symposium Vol. *6*: 385 (1938).

[105] L. G. Barth: Physiol. Zoology *12*: 22 (1939).

[106] V. Bisceglie: Ztschr. f. Krebsforsch. *23*: 340 (1926).

[107] O. Lubarsch; quot. L. Aschoff: Pathologische Anatomie, Fischer, Jena 1928.

[108] C. Sternberg: Wien. klin. Wchnschr. *42*: 1621 (1929).

[109] C. Lewin: Ztschr. f, Krebsforsch, *32*: 126 (1931).

composition products by repeated washing, are injected.[110–112] This seems to indicate that the necrotic tumor parts, inoculated according to the first technique, contain growth promoting substances, and apparently similar products are present in the serum of cancer patients since treatment of isolated tumor cells with cancer serum, prior to transplantation, was found to increase the virulence notably.[111, 113]

Direct investigations of the influence of tumor extracts on the growth of animal tumors have led to conflicting results as to the presence of growth stimulating factors. On the basis of extensive experimental work, Casey [114] reported that Berkefeld filtrates of Brown-Pearce rabbit carcinomas, when injected into rabbits two weeks prior to the inoculation of the same tumor, considerably enhanced the susceptibility of the animals to the tumor, increased the tumor yield, and stimulated the growth rate. Formation of metastases occurred more frequently in treated animals and their duration of life was shortened. It is noteworthy that the Brown-Pearce carcinoma filtrates had no influence on the growth of transplanted mouse carcinomas, nor did extracts of the mouse tumors affect the growth of the rabbit carcinomas. Furthermore, it is of interest, in view of divergent findings mentioned later, that some experiments which the same author performed with tumor filtrates resulted in increased resistance of rabbits to Brown-Pearce carcinoma.[115] In continued work,[116] it was demonstrated that the tumor promoting substance prepared from

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110. N. Waterman: Ztschr. f. Krebsforsch. *27*: 228 (1928).
111. K. Stern & R. Willheim: Ztschr. f. Krebsforsch. *39*: 54 (1933).
112. G. Witte: Deutsche Ztschr. f. Chir. *245*: 251 (1935); Ztschr. f. Krebsforsch. *42*: 340 (1935).
113. H. Hirschfeld: Ztschr. f. Krebsforsch. *11*: 388 (1912).
114. A. E. Casey: Proc. Soc. Exper. Biol. & Med. *29*: 816 (1932); *30*: 674; 1025 (1933); *31*: 663; 666 (1934); *40*: 223; 228; 230 (1939); Am. J. Cancer *21*: 760; 776 (1934); *22*: 665 (1934); *31*: 446 (1937).
115. A. E. Casey: Proc. Soc. Exper. Biol. & Med. *34*: 111 (1936).
116. A. E. Casey: Am. J. Cancer *35*: 354 (1939).

Brown-Pearce carcinoma was not identical with the "spreading factor" of bacterial infection, extracted from testis by Duran-Reynals,[117] and that the material lost its activity after heating.[118] The author recently presented his findings in a detailed review.[119] Similar effects, stimulating the tumor growth in animals treated with tumor extracts, have been obtained by other authors,[120] and the growth of Ehrlich-Putnoky rat carcinoma was found accelerated when the animals were injected with extracts of the same tumor inactivated by heat.[121] Alcoholic extracts of transplanted mouse carcinomas were shown to stimulate the growth of homologous tumors, whereas alcoholic extracts of heterologous tumors, or normal tissues, exerted a growth inhibiting effect.[122] Since this partly agrees with the aforementioned findings, it may be assumed that the growth stimulation is due more to nutritive material common to the same species than to substances specific for malignant tumors. However, the growth stimulating property of homologous tumor material has not been observed by other investigators. In one study, extracts of Brown-Pearce carcinoma failed to show any effect on the growth of subsequently inoculated tumors,[123] and other workers even observed increased resistance of animals to tumor inoculation when they had been previously treated with extracts of homologous tumors (Ehrlich mouse carcinoma, Jensen rat sarcoma, Brown-Pearce rabbit carcinoma). These extracts had been prepared by freezing and thawing tumor tissues, and it was stressed that the active factors were thermolabile. Furthermore, it is noteworthy that the same extracts which increased the resistance of animals to tumors, when the treatment was performed prior to the tumor inoculation, definitely stimulated the tumor growth when administered to animals previously inoculated with tumors.[124] A confirmation of the essentials of these experiments has been presented.[125] Hence, the time factor appears to be of great importance for the outcome of such experiments and, in addition, the method of administration, and the dosage of the extracts employed, may determine their effect. Repeated intratesticular injections of Berkefeld filtrates of Brown-Pearce rabbit carcinoma appeared to make rabbits more susceptible to subsequent tumor inoculation, whereas subcutaneous injections of higher dilutions of the filtrates increased the resistance of the animals and prolonged their life.[126] Thus, the action of tumor extracts on neoplastic growth obviously depends on a number of factors, perhaps only partly accounted for at present, and deviations in the experimental arrangements may explain the divergent results recorded. Another approach for testing neoplasms for the presence of growth stimulating factors has been the addition of extracts of Rous sarcoma to tissue cultures of normal fibroblasts. When compared with the effect of extracts of the normal adult chicken muscle, the tumor extracts exhibited a somewhat weaker activity, as measured by the acceleration of growth of the fibroblasts and, accordingly, the authors denied the validity of assumptions which claim for tumor tissue an abundant content of growth promoting substances.[127] Further observations made by the authors of the preceding study pointed to a very short latent period of the growth of neoplastic (Rous sarcoma) tissue cultures, as compared with the behavior of normal adult, or embryonic, chicken tissue. This fact was considered to indicate not so much an increased presence of growth factors in the malignant tumors, as to reflect the lack of organization and the independence from regulating influences inherent in neoplastic tissue.[128] The influence of tumor extracts on plant growth has also been studied; extracts of normal tissue, benign and malignant tumors were found to exhibit the same qualities, i.e., of inhibiting germination and growth of watercress seeds.[129] A definite evaluation of these observations pertaining to biologically heterogeneous phenomena appears very difficult, and the same applies to some extent to reports on effects obtained, respectively, with the carcinolytic, and the antilytic factors (cf. chapter X). The carcinolytic factor was seen to impede the growth and metamorphosis of tadpoles, whereas the antilytic factor enhanced these processes.[130] The same antagonism between the action of the two factors was observed in the germination of oats,[131] while only the antilytic factor, but not the carcinolytic one, stimulated the germination of watercress seeds.[132] The assumption may be derived from this that the antilytic factor present in the body of tumor hosts is endowed with a property stimulating growth, becoming manifest in various biological phenomena.

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[117] F. Duran-Reynals: Compt. rend. Soc. de biol. *99*: 6 (1928); J. Exper. Med. *50*: 327 (1929); *61*: 617 (1935); Ann. Inst. Pasteur *57*: 597 (1936).

[118] A. E. Casey & V. Moragues-Gonzales: Am. J. Cancer *38*: 59 (1940).

[119] A. E. Casey: Cancer Research *1*: 134 (1941).

[120] L. Spadafina: Cancer, Bruxelles *11*: 1 (1934).

[121] J. Putnoky & M. Háry: Ztschr. f. Krebsforsch. *46*: 30 (1937).

[122] V. Carminati: Tumori n. s. *12*: 319 (1938).

[123] W. Raab: Ztschr. f. Krebsforsch. *46*: 343 (1937).

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[124] G. Domagk & Ch. Hackmann: Ztschr. f. Krebsforsch. *42*: 192 (1935); Zentralbl. f. Path. *63*: Erg. H., 116 (1935).

[125] C. Dittmar: Ztschr. f. Krebsforsch. *45*: 109 (1937).

[126] H. Malluche: Beitr. z. klin. Chir. *167*: 481 (1938).

[127] R. S. Hoffman, E. Tenenbaum & L. Doljanski: Nature, London *144*: 1092 (1939).

Problems of very far reaching implication have been attacked in studies in which substances of biological origin were tested as to their properties to stimulate, or to inhibit, the growth of normal, or neoplastic tissues. The establishment of a growth promoting action exhibited by biological substances of known nature may reveal significant aspects of the tumor process and the possible exclusion, or neutralization of such substances would represent a useful approach for practical purposes. On the other hand, it is obvious that therapeutic measures might be made directly available by the disclosure of substances restraining tumor growth. Valuable information might be gained from studies with both groups of growth active substances, inasmuch as they were to render possible differentiation between normal and neoplastic growth—that is, in the event that substances could be detected which influence only one of the two types of growth, whereas the other one would not be affected, or be affected in the opposite direction. Because such findings may eventually yield clues for a better understanding of the regulation of physiologic growth, an accomplishment which so far has been very insufficiently realized, they doubtless represent an extremely important requirement for the solution of the problem exhibited by neoplastic growth processes.

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[128] L. Doljanski & R. S. Hoffman: Nature, London *145*: 857 (1940).
[129] B. Lustig & H. Wachtel: Compt. rend. Soc. de biol. *132*: 246 (1939).
[130] K. Pichler: Klin. Wchnschr. *14*: 1857 (1935).
[131] K. Pichler: Klin. Wchnschr. *15*: 781 (1936).
[132] E. Freund & B. Lustig: Wien. klin. Wchnschr. *50*: 1662 (1937).

The exploration of growth-promoting substances has included several endocrine products, and for this reason these aspects have been discussed in chapter VII; but it may be stressed once more that none of the findings obtained furnished definite evidence of a different reaction of normal and of neoplastic growth in response to administration of hormones. Furthermore, a number of studies clearly demonstrated that various organs, from which growth-stimulating substances can be isolated, contain at the same time also growth-inhibiting principles, and hence it is understandable that divergent results as to the influence on tumor growth have been occasionally reported, in accordance with variations in the technique employed for the extraction of the active substances. A successful separation of two antagonistic factors has been carried out for the lactating mammary gland of rabbits and of cows. Whereas it will be stated later that these tissues were found to contain tumor growth inhibiting factors, also agents promoting growth have been isolated from them, and it was remarked that the latter are present in the aqueous extract of the fraction of the tissue soluble in ether, whereas the former was contained in the part insoluble in ether.[133, 134] Similarly, embryonic extracts possess, in addition to growth inhibiting factors discussed later, agents promoting tumor growth since injections of chick embryo extracts into mice treated with benzpyrene slightly increased the tendency to tumor development.[135] In chapter V, experiments were enumerated, in which the influence of feeding various organs and tissue extracts on tumor development and growth has been studied. It is obvious that an important part is played in the maintenance of all types of growth by certain vitamins, and their relations pertaining to tumor growth have been detailed in chapter V. Recently great interest has been focused on biotin, a highly potent growth factor contained in the vitamin B complex. Preliminary work suggested the presence of increased biotin concentrations in malignant tumors,[136] but continued studies of a broader scope yielded results which made it unlikely that a primary connection exists between neoplastic growth and the biotin content of tumors.[137] Hence it is questionable whether the proposal to utilize avidin, viz., the biological antagonist of biotin, for cancer therapy,[138] may be regarded as exhibiting promising aspects. In this connection reference must be made to a study which indicated a cocarcinogenetic effect of biotin in the induction of hepa-

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133. J. B. Murphy: 2nd Internat. Cancer Congress, Brussels *1*: 379 (1936).
134. D. A. MacFadyen & E. Sturm: Science *84*: 67 (1936).
135. J. F. Riley & A. B. Wallace: Brit. J. Exper. Path. *22*: 24 (1941).
136. P. M. West & W. H. Woglom: Science *93*: 525 (1941).
137. P. M. West & W. H. Woglom: Cancer Research *2*: 324 (1942).
138. W. L. Laurence: Science *94*: 88 (1941).

tomas by means of butter yellow ([139] cf. chapter V), but doubtlessly additional work will be required for clarifying the problem as to whether biotin and avidin represent growth factors of primary importance for the neoplastic process.

In view of the importance assigned to chronic irritation in connection with neoplasia, particular significance may be attributed to experiments in which a growth promoting activity has been demonstrated as being inherent in products of cellular injury. For such purposes, inflammatory exudates were used, and their repeated injection into ears of rabbits was followed by a marked proliferative reaction.[140] Yet this report must be considered preliminary, the growth-promoting quality of these substances having not been tested as yet in direct connection with transplanted or chemically induced tumors. Growth factors present in urines of humans have been investigated, and were measured by the effect on the growth of a protozoon (colpidium campylum); both stimulating and inhibiting substances were isolated from urines, but a prevalence of the latter substances was seen in normal persons, whereas the former prevailed in urines of persons affected with proliferative processes (pregnancy, granulations, malignant tumors).[141] Similar substances were extracted from various tissues, and the highest concentration of factors stimulating the growth of the protozoa were found in liver, spleen and neoplasms.[142] Injections of these substances into mice did not prove them to be carcinogenic, but they caused an endothelial reaction in the liver, spleen and lymph nodes of the animals.[143] Other workers found that a colloidal fraction of urinary substances, which are insoluble in fifty per cent alcohol, lowered the resistance of rats against inoculation of Walker carcinoma 256, but since this effect did not extend to other tumors,[144] its significance appears limited and reference is made to statements on the action of urinary endocrine factors in chapter VII.

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[139] V. du Vigneaud, J. M. Spangler, D. Burk, C. J. Kensler, K. Sugiura & C. P. Rhoads: Science *95*: 114 (1942).

[140] V. Menkin: Cancer Research *1*: 548 (1941).

[141] G. L. Rohdenburg & S. M. Nagy: Am. J. Cancer *28*: 66 (1937).

[142] G. L. Rohdenburg & S. M. Nagy: Am. J. Cancer *30*: 335 (1937).

[143] G. L. Rohdenburg & S. M. Nagy: Am. J. Cancer *30*: 512 (1937).

Substances capable of inhibiting the growth of malignant tumors have been investigated very frequently and reference is made to hormones to which such a property has been ascribed (cf. chapter VII). In addition to true endocrines, some other organs have been shown to contain agents which inhibit tumor growth. Numerous reports of this kind referred to placenta,[145-148] however, some authors reported that placenta extracts impeded the growth of tumors only in adult animals, whereas tumors inoculated into immature animals were even stimulated.[149] Another study showed only a slight inhibitory effect of placenta extracts on rat sarcomas.[150] By using special methods, other authors isolated substances from placenta and embryonic skin of mice which markedly inhibited the growth of mouse carcinomas,[151] but higher concentrations of such factors were found by these authors in prelactating mammary glands of rabbits and cows, and it may be recalled that the growth-inhibiting agents could be separated from the growth-stimulating ones by fractionation with ether.[133, 134] In continued work, it was ascertained that the growth-inhibiting substances are present in the protein fractions of mammary tissue of pregnant rabbits, and may be prepared by saturating the aqueous tissue extracts with ammonium sulfate. However, this product was found effective only in the Bashford mouse carcinoma 63 and did not extend to the Crocker mouse sarcoma 180.[152] Other aspects have been detected by a worker who employed alcoholic extracts of normal and of neoplastic tissues. Subcutaneous injection of these products into tumor-bearing mice was found to inhibit the tumor growth when heterologous extracts were employed, and embryonic tissue extracts proved most effective. It is noteworthy that the inhibition of tumor growth was accompanied by a loss of total body weight, i.e., general damage to the health, which might have been responsible for the impeded tumor growth. On the other hand, as mentioned before, alcoholic extracts of homologous tissue,—whether normal or neoplastic,—enhanced the tumor growth without affecting the total body weight.[122] The effect of serum on tumor growth has been studied by immersing fragments of Flexner-Jobling rat carcinoma in human or animal sera, prior to the tumor inoculation. When the immersion in sera was performed at 37° C, a marked inhibition of the subsequent tumor growth ensued, but this failed to occur when tumor and sera were brought together at 4–5° C. Sera of tumor-bearing rats acted in the same manner as sera of tumor-free animals, yet sera of cancer patients appeared to be less growth inhibiting than normal sera,[153] a finding which might be related to the carcinolytic phenomenon (cf. chapter X). In a similar manner, the influence of extracts of various organs of rats on the growth of mouse sarcoma 180 and of Flexner-Jobling rat carcinoma was tested. That is, tumor fragments were immersed prior to being transplanted in Locke-Ringer extracts of these organs; spleen extracts exerted the greatest inhibition of subsequent tumor growth, but no difference was noted between the action of spleen extracts of normal, tumor-bearing, or "immune" rats.[154]

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[144] F. Bischoff & M. L. Long: Am. J. Cancer *31*: 58 (1937).

[145] F. Bischoff & L. C. Maxwell: J. Pharmacol. & Exper. Ther. *42*: 387 (1931).

[146] L. Gross & S. Wajda: Ztschr. f. Krebsforsch. *37*: 416 (1932).

[147] G. van der Schueren: Proc. Soc. Exper. Biol. & Med. *32*: 1521 (1935).

[148] A. C. Magian: J. State Med. *41*: 657 (1933); abstr. Ztschr. f. Krebsforsch. *40*: Ref. 138 (1934).

[149] V. Baroni & O. Comsia: Compt. rend. Soc. de biol. *113*: 1310 (1933).

[150] P. Maddalena: Clin. Chir. n.s. *12*: 790 (1936); abstr. Ztschr. f. Krebsforsch. *45*: Ref. 298 (1937).

[151] J. B. Murphy & E. Sturm: J. Exper. Med. *60*: 293; 305 (1934).

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[152] D. A. MacFadyen, E. Sturm & J. B. Murphy: J. Exper. Med. *70*: 475 (1939).

[153] K. Sugiura & S. R. Benedict: Am. J. Cancer *27*: 712 (1936).

Tumor growth inhibiting substances have been isolated from beef brain, bone marrow and thymus, by means of ether extraction and precipitation with calcium chloride. The preparations obtained markedly impeded the formation of tar cancer in mice.[155] It will be recalled that similar products were employed by Maisin in his organo-therapy. Mesenchymal tissue has been demonstrated to contain tumor-growth-inhibiting substances, inasmuch as preparations from human rectus sheaths inhibited the growth of transplanted rat tumors,[156] and hydrolysates or autolysates of ox heart muscle were reported to cause regressions of transplanted tumors. In the latter instance amino acids were believed to represent the active agent.[157] A recent study confirmed the inhibiting effect exerted by muscle extracts on transplanted and spontaneous mouse tumors. Several compounds were isolated from dialysates of these muscle extracts, and among them ethanolamine and cadaverine hydrochloride were found to possess the greatest activity in inhibiting the tumor growth.[157a] On the other hand, fatty acids of unsaturated character were recognized as being responsible for a considerable growth restriction of Rous sarcoma of fowl resulting from the administration of pig pancreas extracts.[158, 159] Very interesting results have been gained from studies on growth inhibiting substances which were found present particularly in liver tissue. These substances could be freed from their adsorption to colloids by treatment with alcohol, and one part of them was identified to be aliphatic amines. These agents very effectively inhibited the growth of normal tissue, but the growth of malignant cells remained wholly unaffected. In some experiments the inhibition of normal growth was reversible and apparently did not interfere with other cell functions.[160] Slight inhibition of the growth of mouse sarcoma, which resulted from injections of these substances, were regarded as probably being due merely to the inanition of the animals, since no histological changes were detected in the tumors. One of the growth inhibiting factors was found to be the phosphoric ester of ethanolamine,[161] and it is recalled in this connection that this compound was detected in malignant tumors, while it was absent in most normal tissues.[162] In continuation of this work it was observed that this ethanolamine ester and many other aliphatic amines (excluding diamines and amino acids) strongly inhibited in tissue cultures the growth of normal cells, and caused a vacuolation of the cells, whereas malignant cells were affected neither morphologically nor in their growth.[163] This phenomenon appears highly significant inasmuch as it represents an impressive instance of the existence of chemically defined compounds which affect normal and neoplastic growth in a different manner, and the possibility has been considered that these substances may act as regulators of normal growth.[163]

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[154] K. Sugiura: Am. J. Cancer *32*: 126 (1938).

[155] C. Ronsse: Cancer, Bruxelles *12*: 319 (1935).

[156] J. J. Morton & D. N. Beers: J. Exper. Med. *61*: 59 (1935).

[157] A. H. Roffo: Bull. Assoc. franç. p. l'étude du cancer *26*: 602 (1937); Lancet *2*: 184 (1938).

[157a] E. Boyland: Biochem. J. *35*: 1283 (1941).

[158] O. M. Helmer: J. Exper. Med. *64*: 333 (1936).

[159] O. M. Helmer & G. H. A. Clowes: Am. J. Cancer *30*: 553 (1937).

A tumor-inhibiting activity has been reported for tonsil extracts,[164] and a growth-regulating function and a connection of this organ with the tumor process has been assumed to exist,[165] but the elaboration of this problem has not advanced sufficiently to warrant any definite conclusion. The presence of substances in urines which stimulate the growth of protozoa, and also affect normal tissue growth, has been mentioned before,[141–143] and in another study a slight inhibition of the formation of chemically induced tumors was observed after injection of human urine extracts into mice. The same treatment, however, failed to influence the growth of spontaneous and transplanted mouse tumors.[166]

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[160] A. M. Brues, Y. Subbarow & J. C. Aub: 3rd Internat. Cancer Congress, Atlantic City, 1939.

[161] A. M. Brues, Y. Subbarow, E. B. Jackson & J. C. Aub: J. Exper. Med. *71*: 423 (1940).

[162] E. L. Outhouse: Biochem. J. *30*: 197 (1936); *31*: 1459 (1937).

[163] A. M. Brues & E. B. Jackson: Cancer Research *1*: 557 (1941).

[164] O. Voss: Klin. Wchnschr. *8*: 1098 (1929).

Substances foreign to the animal body, viz., the auxins of plants (phytohormones), were tested as to their influence on tumor growth, but were found ineffective.[167, 168] One investigator reported on a growth inhibition of Bashford mouse carcinoma following injections of one phytohormone, namely, potassium β-indole acetate,[169] but most likely this effect depended on the general toxic quality of the substance employed. As mentioned in chapter II, the heteroauxins β-(Indole-3)-propionic acid and γ-(Indole-3)-n-butyric acid were found to be noncarcinogenic for mice.[169a] Interesting results were obtained by studying the effect of the fraction of Bacillus proteus filtrates which is insoluble in alcohol. This product notably inhibited the growth of tissue cultures of mouse sarcoma 180, while affecting normal cells to a smaller degree,[170] and this may be connected with the Shwartzman phenomenon, an action of bacterial filtrates discussed in chapter VIII. Recently, tumor-growth-inhibiting qualities were found present in yeast extracts, the subcutaneous, or intravenous, injection of which caused complete disappearance of eight out of thirty-three spontaneous mammary cancers of the mice treated.[171] Further work demonstrated a growth inhibiting and curative effect of yeast extracts in spontaneous and transplanted mouse carcinoma. The active factors were described as water-soluble, of non-protein nature, and as being apparently not identical with any of the known constituents of the vitamin B complex.[172] Intravenous injection of the yeast extracts administered to mice ten days prior to the transplanting of the carcinoma prevented tumor development in about twenty-one per cent, and addition of riboflavin to the yeast extract increased its effectiveness whereas riboflavin alone showed only slight activity.[173] No definite explanation can now be offered as to the chemical nature of the antiblastic factor present in these yeast extracts; other investigations, which demonstrated yeast feeding as a measure capable of counteracting certain carcinogenic stimuli (butter yellow), have been discussed in chapter V, but these effects seem to involve different processes.

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[165] S. Peller: Wien. med. Wchnschr. *85*: 682 (1932); Wien. klin. Wchnschr. *49*: 796 (1936).

[166] F. C. Turner: Pub. Health Rep. *54*: 1855 (1939).

[167] F. Kögl: Ztschr. f. Krebsforsch. *40*: 203 (1934).

[168] C. Dittmar: Biochem. Ztschr. *279*: 99 (1935).

[169] A. Tanaka & S. Tuboi: Gann *34*: 354 (1940).

[169a] M. J. Shear & J. Leiter: J. Nat. Cancer Inst. *2*: 241 (1941).

[170] L. C. Fogg: Pub. Health Rep. *51*: 56 (1936).

3. *Virus Tumors.*

Experimental evidence has established beyond any doubt that certain neoplastic growths are caused by specific agents of a virus nature. Among these, malignant sarcomas of fowl, first described and studied by Rous, are the most familiar and most widely investigated example. Yet a sharp division of opinion must be recorded as to the theoretical conclusions which have been drawn from the experimental observations. On one hand, a number of authors felt justified to draw gen-

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171. R. Lewisohn, C. Leuchtenberger, R. Leuchtenberger & D. Laszlo: Proc. Soc. Exper. Biol. & Med. *43*: 558 (1940).

172. R. Lewisohn, C. Leuchtenberger, R. Leuchtenberger, D. Laszlo & K. Bloch: Cancer Research *1*: 799 (1941).

173. R. Lewisohn, C. Leuchtenberger, R. Leuchtenberger, D. Laszlo & K. Bloch: Science *94*: 70 (1941).

eral conclusions from these instances of animal neoplasia, inasmuch as they regarded all malignant tumors as being caused by a virus infection, and thus offered a definite answer to the much debated problem of cancer etiology. However, the majority of oncologists have refused so far to accept this view of considering neoplasia in general as being due to specific viruses, or at least they expressed well-founded doubts as to whether such a theory had been convincingly proved. The writers do not believe it possible at present—and even would think it presumptuous—to offer a clear-cut decision as to whether it would be possible, on the basis of further work, to establish a general virus etiology of malignant tumors, or whether such a theory will be recognized as definitely untenable. Findings, which may be regarded as supporting one or the other of these two views, will be discussed, and experimental work, dealing with the biochemical aspects of the established virus tumors will be placed in the foreground of this presentation.

The experimental criterion for a virus etiology of a neoplasm may be seen in the fact that it can be transmitted by cell-free filtrates, or extracts of this tumor, and that the resulting tumors closely resemble the original neoplasm in morphological and biological respects. A number of fowl sarcomas have been found to exhibit this behavior, and for details of their pathology reference is made to recent reviews on virus tumors of fowl[174, 175] and to a survey on the pathology of virus tumors in mammals.[176] Since, in these instances, the complete destruction of the cellular elements of the tumors by desiccation, freezing, or mechanical measures or the exclusion of all tumor cells by employing definitely cell-free

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[174] P. Rous: Am. J. Cancer *28*: 233 (1936).

[175] J. Engelbreth-Holm: Acta path. et microbiol. Scandinav. Suppl. *38*: 27 (1938).

[176] C. G. Ahlström: Acta path. et microbiol. Scandinav. Suppl. *38*: 37 (1938).

extract or filtrates did not prevent this material from reproducing neoplasms in animals to which it was administered, it had to be assumed that an agent, which can be separated from cellular elements, is responsible for this type of neoplasia. Since this agent, furthermore, appeared to multiply in the organism, it appeared most likely to be of a virus nature. Much work has been devoted to the determination of the physical and chemical qualities of this tumor producing agent.[cf. 175] It was demonstrated that the Rous sarcoma agent is ultrafilterable and, accordingly its diameter was assumed to be smaller than 0.1 micron.[177] More precise data were furnished by other workers who determined the particle diameter of the Rous sarcoma virus by means of a centrifugal method as sixty to seventy millimicrons and, for comparison, it may be mentioned that the size of the vaccinia virus was found to be one hundred seventy to one hundred eighty millimicrons, the size of influenza virus to be eighty-seven to ninety-nine millimicrons.[178] Spectrographic analysis of the perfusion fluid of fowl tumors was reported to show a parallel between the tumor producing activity and the intensity of an absorption band situated at 2000 Ångstrom. Chemically, a protein containing phosphorus was found in this perfusion fluid, but since the tumor producing factor proved diffusible, it was supposed that the protein was merely a colloidal carrier of the active agent.[179] Cell-free aqueous extracts of Rous sarcoma have been adsorbed to inorganic colloids, such as kaoline or iron hydroxide, and could be eluted from these adsorbents by means of alkaline solutions. Both the adsorbates and the elutions proved capable of producing tumors.[180]

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[177] H. Yaoi & W. Nakahara: Gann *29*: 222 (1935).

[178] W. J. Elford & C. H. Andrewes: Brit. J. Exper. Path. *17*: 422 (1936).

[179] F. Pentimalli: Tumori n.s. *10*: 14; 327 (1936).

[180] E. Maschmann & B. Albrecht: Ztschr. f. physiol. Chem. *196*: 241 (1931).

Similar results have been obtained by employing dyes insoluble in water as adsorbents,[181] and because of these findings a number of authors have been inclined to ascribe an enzymatic nature to the Rous sarcoma agent.[182-185] Furthermore, it has been reported that the Rous sarcoma agent could be inactivated by treatment with pancreas extract, but the factor responsible for this effect could not be identified with any of the known pancreatic enzymes (diastase, trypsin, lipase).[186]

Chemical experiments showed the fowl sarcoma agent as being sensitive to oxidation which destroys its activity. Addition of cysteine,[187] prussic acid,[188] or of sodium bisulfite[189] proved capable of preventing the oxidation and of increasing the stability of the agent. More recently, similar relations were established for the agent of fowl leucosis and the possibility of reducing and reactivating the oxidized fowl tumor agent by means of cysteine was demonstrated.[190] Some authors believed they had found evidence for a lipid nature of the chicken tumor agent, and reported they had transmitted the tumors by means of injections of lipid tumor extracts.[191, 192] This has been contested, since acetone or carbon tetrachloride extracts of Rous sarcoma failed to pro-

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181. E. Fränkel: Ztschr. f. physiol. Chem. *200*: 126 (1931).
182. Teuschlaender: Ztschr. f. Krebsforsch. *24*: 223 (1927).
183. E. Fränkel: Ztschr. f. Krebsforsch. *25*: 407 (1927).
184. F. Pentimalli: 1st Internat. Cancer Congress, Madrid *2*: 81 (1933).
185. F. Blumenthal: Ergebnisse der experimentellen Krebsforschung und Krebstherapie, Leiden 1934.
186. A. Pirie: Biochem. J. *27*: 1894 (1933).
187. J. H. Mueller: J. Exper. Med. *48*: 343 (1928).
188. W. E. Gye & W. J. Purdy: Brit. J. Exper. Path. *11*: 282 (1930).
189. A. Pirie & B. E. Holmes: Brit. J. Exper. Path. *12*: 127 (1931).
190. J. Engelbreth-Holm & O. Fredericksen: Acta path. et microbiol. Scandinav. Suppl. *37*: 138 (1938).
191. J. W. Jobling & E. E. Sproul: Science *84*: 229 (1936).
192. J. W. Jobling, E. E. Sproul & S. Stevens: Am. J. Cancer *30*: 667; 685 (1937).

duce tumors,[193] and it was assumed that lipid extracts of fowl tumors caused tumor formation only when contaminated by minute particles of the dried tumor material used for the extraction.[194] Likewise, other workers found the powder residue of Rous tumors after extraction with pentane or chloroform-pentane mixtures still active, and assumed a protein nature of the virus. This view appears to be supported by the finding that heating of dried tumor did not impair its activity, whereas the same material was inactivated when heated in the presence of water, the latter procedure apparently denaturing the proteins.[195] Later analyses of purified chicken tumor agent strongly suggested that nucleic acids represent the active factor,[196] an assumption which is supported by continued work to be mentioned. Some experiments seemed to indicate that the lipid fraction of the Rous sarcoma agent contained an inhibiting factor, the removal of which by means of acetone, or carbontetrachloride extraction, increased the biological activity of the material.[197] However, another investigator ascribed a stabilizing effect to the lipid content of Rous sarcoma material, and the active constituent of the agent which had been purified by centrifugation and tryptic digestion was believed to be a nucleoprotein; a treatment with formaldehyde, which reduced the number of amino groups, was found to decrease the activity of the agent.[198] In recent studies a characteristic spectrum of ultraviolet absorption was established for purified active agent of fowl tumors.[cf. 179] This spectrum indicated the presence of purine and pyrimidine bases, fifteen per cent of which could be isolated as nucleic acids. These authors regarded a nucleoprotein as the active principle and found it to be inactivated by ultraviolet irradiation.[199] Chemical analysis of purified agent of Rous sarcoma I showed the presence of thymonucleic acid and a nitrogen content of 8.9 per cent. Various physical constants of this agent were determined; tumor development resulted from the injection of 10^{-7} to 10^{-9} grams of this material, and the agent was found to inhibit in vitro the activity of cytochrome oxidase and of catalase.[200]

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[193] E. M. Fraenkel & C. A. Mawson: Brit. J. Exper. Path. *18*: 454 (1937); Nature, London *139*: 282 (1937).

[194] A. Pollard & C. R. Amies: Brit. J. Exper. Path. *18*: 198 (1937).

[195] M. Levine & E. J. Baumann: Proc. Soc. Exper. Biol. & Med. *36*: 820 (1937).

[196] A. Claude: Science *87*: 467 (1938).

[197] E. M. Fraenkel: Tumori n.s. *12*: 114 (1938).

[198] A. Pollard: Brit. J. Exper. Path. *20*: 429 (1939).

[199] A. Claude & A. Rothen: J. Exper. Med. *71*: 619 (1940).

It is implied by some of the aforementioned studies that the activity of the filterable tumor agents could be considerably increased by various procedures resulting in their liberation from inactive or inhibiting material. High speed centrifugation has been most frequently employed,[201–203] sometimes combined with tryptic digestion.[198] Freezing to minus 60°C has been found to increase the stability of fowl sarcoma virus isolated by high speed centrifugation, and, to a lesser degree, addition of sodium sulfate helped to preserve the activity of the virus.[204] Other workers reported on a concentration and purification of Rous sarcoma agent, which was achieved by a digestion of the tumor material by a polysaccharide enzyme isolated from pneumococci. This procedure did not destroy the activity of the agent, and it could be precipitated by means of basic proteins and separated from them by means of electrophoresis.[205]

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[200] K. G. Stern & F. Duran-Reynals: Science *89*: 609 (1939).

[201] J. McIntosh: J. Path. & Bact. *41*: 215 (1935).

[202] A. Claude: J. Exper. Med. *66*: 59 (1937); Am. J. Cancer *30*: 742 (1937).

[203] A. Pollard: Brit. J. Exper. Path. *19*: 124 (1938).

[204] E. A. Kabat & J. Furth: J. Exper. Med. *71*: 55 (1940).

[205] D. Shemin, E. E. Sproul & J. W. Jobling: J. Exper. Med. *72*: 697 (1940).

As to the biological properties of the tumor viruses, it is noteworthy that precise data have been furnished, demonstrating a widespread presence of the Rous sarcoma agent in the body of the animals seven days after its transmission.[182, 206] Furthermore, the Rous sarcoma virus has been shown to be active in vitro; namely, tissue cultures of normal chickens fibroblasts were apparently transformed into malignant cells when the virus was added, since re-inoculation of such tissue cultures resulted in tumor development. However, an infection of human macrophages and monocytes with the virus failed to yield a material capable of tumor production.[207] A somewhat different experimental arrangement was used in another study in which tissue cultures of Rous sarcoma were exposed to β-radium radiation. This treatment killed the cells, but the virus was found still active, since added normal fibroblasts became malignant and produced tumors after injections into fowl.[208] A speculative theory was proposed which considered the Rous sarcoma agent not as an extrinsic virus, but supposed it to be derived from mitochondria which had been freed from cells and had acquired some new qualities of an, as yet, undefined nature; when such mitochondria were to be phagocyted by normal cells, they would transform them into malignant cells and thus give rise to development of neoplasms.[209] An inhibiting factor of tumor growth has been isolated from slowly growing chicken tumors; this principle could be separated from the tumor-producing agent and was believed to be of protein nature.[210] In connection with the chemical characterization of the fowl tumor agent previously described, it is significant that a fraction has been isolated from chick embryo which exhibited

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[206] E. Mellanby: J. Path. & Bact. *47*: 47 (1938).
[207] R. J. Ludford: Am. J. Cancer *31*: 414 (1937).
[208] L. Halberstädter & L. Doljanski: Nature, London *143*: 288 (1939).
[209] A. Graffi: Ztschr. f. Krebsforsch. *50*: 196; 501 (1940).
[210] A. Claude: Am. J. Cancer *37*: 59 (1939).

the same physical and chemical qualities as the purified activ fraction of chicken tumors, but in contrast to the latter product, the embryo extract failed to induce tumors.[211] Hence i appears very likely that in spite of the detailed knowledg available on the fowl tumor agent, the chemical and physica data do not define its nature completely, but it may be assumed that the tumor-producing activity is linked with som biological qualities as yet unknown

Valuable information supplementing the analytical findings has been gained from immunological studies of viru tumors and, as remarked in chapter VIII, this part of tumo immunology appears to exhibit more easily discernible aspects than those pertaining to other types of neoplasia. Th presence of specific antibodies in the sera of fowl resistan to transmission of Rous sarcoma has been made very probable, since such sera were found to neutralize in vitro th activity of the Rous sarcoma agent.[212] Similarly, an anti-fowl serum, obtained from goats, has been shown to contai neutralizing factors, inasmuch as interaction of this serum with chicken tumor agent in the presence of complement wa followed by a loss of the tumor producing activity.[213] Furthermore, it was reported that parallel to the aging o chickens, a factor increases in their blood which suppresses the tumor-producing action of chicken tumor viruses, and the susceptibility of the animals to these tumors was believed to depend on the concentration of this neutralizing facto in their blood.[214] Immunological investigations performed with purified Rous sarcoma agent showed the presence of two antigens in the substrate, which can be separated, and injection of one of these antigens into rabbits produced immune sera which contained tumor inhibiting antibodies.[215] It is very significant that the tumor producing "heavy fraction" isolated from Rous sarcoma has been differentiated by immunological methods from analogous chicken spleen, or embryo fractions. Although chemically these fractions appeared identical, the tumor fraction was found to contain a thermostable heterogenetic antigen plus a thermolabile species-specific antigen, which latter was lacking in the spleen fraction.[216] While complement fixation tests failed to differentiate between the heavy material obtained from spleen and that derived from chicken sarcoma,[217] these fractions proved biologically of different activity. Heavy material of chicken tumors, when injected into rabbits, produced antibodies which neutralized the fowl tumor agent, whereas similar material prepared from chicken spleen failed to evoke such antibodies.[218] Likewise, it has been found possible to differentiate, by means of precipitin tests, between antigenic properties of highly purified chicken tumor virus and the qualities of analogous substrates prepared from normal chicken tissue.[219] An immunological relationship was assumed to exist between true virus tumors and tumors of fowl induced by dibenzanthracene, although the latter could not be transmitted by means of filtrates. However, injection of filtrates, or pulp of the dibenzanthracene tumors, into rabbits, resulted in sera which neutralized the tumor producing activity of Rous sarcoma agent,[220, 221] and complement fixation tests were interpreted as indicating the presence of a common antigen in virus tumors and in dibenzanthracene tumors of fowl.[222]

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[211] A. Claude: Proc. Soc. Exper. Biol. & Med. *39*: 398 (1938).
[212] F. G. Banting & S. Gairns: Canad. M. A. J. *30*: 615 (1934).
[213] R. Knox: Brit. J. Exper. Path. *20*: 391 (1939).
[214] F. Duran-Reynals: Yale J. Biol. & Med. *13*: 61 (1940).

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215. C. R. Amies & J. G. Carr: J. Path. & Bact. *49*: 497 (1939).
216. J. Furth & E. A. Kabat: Science *91*: 483 (1940).
217. J. Furth & E. A. Kabat: J. Exper. Med. *74*: 274 (1941).
218. E. A. Kabat & J. Furth: J. Exper. Med. *74*: 257 (1941).
219. M. K. Barret: Cancer Research *1*: 543 (1941).
220. L. Foulds: Am. J. Cancer *31*: 404 (1937).
221. L. Foulds & L. Dmochowski: Brit. J. Exper. Path. *20*: 458 (1939).
222. L. Dmochowski & R. Knox: Brit. J. Exper. Path. *20*: 466 (1939).

Yet another worker found that filtrates of dibenzanthracene tumors of fowl could be inactivated only by sera derived from immunization with the same tumor, but not by antisera directed against other filterable fowl tumors and, accordingly, he assumed that the chemically induced tumors possessed biological qualities distinguishing them from other fowl tumors.[223] Immunological studies performed with a mammalian virus tumor, viz., the Shope rabbit papilloma, were reported to prove the virus etiology of the subsequent developing malignant neoplasms, although the latter may not always be transmissible by means of filtrates.[224, 225] Further work demonstrated by means of complement-fixation a different immunological quality of antigens isolated from Brown-Pearce rabbit carcinoma and those prepared from virus tumors of rabbits, although these two types of antigens exhibited some similarities.[226]

Interesting phenomena, which have been regarded as supporting the theory of a general virus etiology of neoplasia, have been observed in the course of investigations employing the combined administration of virus-like agents and of carcinogenic stimuli. When tar warts were induced in rabbits and then Shope papilloma virus was injected into the animals, a fulminant carcinosis frequently resulted, and the administration of the virus appeared in most instances to activate greatly the carcinogenic effect of tar.[220, 221] Independently, similar results were obtained by simultaneous administration of Shope papilloma virus and of tar or benzpyrene to rabbits,[227–232] and injection of virus extracted from human

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223. M. des Ligneris: Compt. rend. Soc. de biol. *121*: 1579 (1936).

224. R. E. Shope & E. W. Hurst: J. Exper. Med. *58*: 607 (1933).

225. J. G. Kidd, J. W. Beard & P. Rous: Proc. Soc. Exper. Biol. & Med. *33*: 193 (1935–36).

225a. P. Rous & J. W. Beard: J. Exper. Med. *62*: 523 (1935).

226. J. G. Kidd: J. Exper. Med. *71*: 335; 351 (1940).

227. J. G. Kidd & P. Rous: Proc. Soc. Exper. Biol. & Med. *37*: 518 (1937); J. Exper. Med. *68*: 529 (1938).

warts (verruca vulgaris) into rabbits was reported to accelerate, in some instances, the cancerization of papillomas which had been induced in the ears of the animals by means of benzpyrene.[233] However, it must be remarked that in one investigation no enhancement of carcinogenesis was found when rabbits were exposed to the combined action of carcinogen and of Shope papilloma virus,[234] a finding which is in contrast to the previously mentioned results and makes further re-examination desirable. Attempts have been made to test for the presence of an agent of virus nature in mouse sarcoma 37 by employing a similar experimental arrangement as had been used in the studies with Shope papilloma virus. Finely mashed and frozen tumor tissue was injected into mice with lesions induced by benzpyrene, but the treatment failed to enhance the malignant transformation of these lesions as it has been observed in the case of the rabbit papilloma virus; [235] hence this negative outcome failed to furnish evidence for the presence of a virus in the mouse sarcoma, although it cannot be considered definite proof against such an assumption.

In addition to the virus tumors of fowl, and the infectious rabbit papillomas, a virus etiology has been advocated for several other types of neoplasia. One of these instances is represented by a malignant tumor of a cold-blooded animal, viz., an adenocarcinoma of the kidney in a "lepard frog";

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228. P. Rous & J. G. Kidd: J. Exper. Med. *67*: 399 (1938); *71*: 787 (1940).

229. C. H. Andrewes, C. G. Ahlström, L. Foulds & W. E. Gye: Lancet *2*: 893 (1937).

230. C. G. Ahlström & C. H. Andrewes: J. Path. & Bact. *47*: 65 (1938).

231. C. H. Andrewes & C. G. Ahlstrom: J. Path. & Bact. *47*: 87 (1938).

232. A. Lacassagne & W. Nyka: Bull. Assoc. franç. p. l'étude du cancer *26*: 154 (1937).

233. A. Brunschwig, D. Tschetter & A. Hamann: Am. J. Cancer *38*: 50 (1940).

234. P. Rondoni & M. J. Eisen: Tumori n.s. *11*: 511 (1937).

235. W. H. Woglom: Am. J. Cancer *35*: 374 (1939).

transplants of this tumor to other frogs underwent resorption, but in twenty per cent of these animals, kidney tumors of identical histology developed six months later, and this phenomenon was believed to be due to the action of a virus contained in the original transplant.[236] A greater number of studies have dealt with filterable fowl tumors which originally had been induced by the action of carcinogens, such as was the case in a fowl sarcoma induced by dibenzanthracene which, after several transplantations, could also be transmitted by means of injections of cell-free filtrates,[228] a result which necessitated the assumption that these filtrates contained a virus-like tumor producing agent. Similarly, other workers observed tumor development in fowl injected with filtrates of fowl tumors induced by tar or dibenzanthracene [237, 238] and in instances where filtrates of fowl sarcomas, induced by these carcinogens, failed to elicit tumor development, their virus etiology was believed very probable since injection of these filtrates produced specific antibodies capable of neutralizing the Rous sarcoma virus.[220, 239] However, recently, extensive experiments were reported which showed that a successful transmission of chemically induced chicken tumors was possible only when intact, living, cells were used, whereas filtrates proved ineffective and also a previous roentgen irradiation of the tumor substrates which was sufficient to damage cellular elements, but not filterable agents of virus nature, destroyed the transplantability.[240] This may lead to the enumeration of some similar experiments on the basis of which an infectious etiology has been held invalid for mammalian tumors. Thus it was reported that the Shope papilloma virus resists high pressure up to four thou-

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236. B. Lucké: J. Exper. Med. *68*: 451 (1938).
237. E. Mellanby: J. Path. & Bact. *46*: 447 (1938).
238. J. McIntosh & F. R. Selbie: Brit. J. Exper. Path. *20*: 49 (1939).
239. C. H. Andrewes: J. Path. & Bact. *43*: 23 (1936).
240. J. B. Murphy & E. Sturm: Cancer Research *1*: 609 (1941).

sand atmospheres whereas, only eighteen hundred atmospheres destroy the transplantability of Brown-Pearce rabbit carcinoma,[241] and similar differences as to resistance against high pressure were observed by comparing mouse tumors and Rous sarcomas.[242, 243] Furthermore, successful transmission of virus tumors of fowl was possible after administration of roentgen doses which definitely destroyed the transplantability of mouse tumors,[244] and all of these findings together have been taken as indication that the mammalian tumors investigated are not transmitted by an agent of virus nature. In this connection it must first be pointed out that so far no convincing evidence has been made available demonstrating a possibility to transmit the common types of mammalian tumors by means of cell-free substrates. Long ago it was reported that the injections of autolysates of mouse tumors into mice was followed by the development of malignant tumors in a small percentage of the animals treated,[245] but subsequent experiments were incapable of reproducing these results, and the assumption that the tumors ensuing from the injection of autolysates were in reality spontaneous appears very plausible.[246] According to a report of more recent date, cell-free extracts prepared from frozen mouse tumors led to formation of histologically identical tumors,[247] but this statement has been contested [248, 249] and could not be confirmed.[250] In particular it has been stressed that exposure of tumor tissue to very low temperatures does not necessarily result in a complete destruction of tumor cells, but that especially resistant small cells can survive this treatment and accordingly tumor formation may be initiated by them upon re-inoculation to animals.[251] The claim has been advanced that in all mammalian tumors, a virus of specific nature is present which can be demonstrated by staining methods, but experiments on tumor production with this virus were not convincing,[252] and no further corroborating evidence has been furnished. It was reported that cell-free filtrates of a mouse sarcoma induced by dibenzanthracene produced, in some instances, tumors of analogous histological type,[253] but a final evaluation of this observation requires additional experimental work along this line. As mentioned before, a virus nature has been ascribed to a mammary-cancer-producing agent present in the milk of high-mammary-cancer-strain mothers.[60] Yet, even if proven, this factor must be regarded as effective only in this special form of neoplasia. Negative results have been obtained in experiments attempting a cell-free transmission of mammalian tumors, such as Brown-Pearce rabbit carcinoma, mouse ascites carcinoma, and human neoplasms.[254] In evaluating experiments frequently recorded, according to which mammalian tumors have been transmitted by blood, or normal organs of tumor hosts, it was pointed out that these findings apparently must be explained by the presence of tumor cells in the substrates used. But in reviewing the literature no convincing evidence was found proving a reproducible transmission of mammalian tumors by means of cell-free material.[255] Finally, negative conclusions as to an infectious etiology of mammalian tumors have been derived from morphological studies on transplants of mouse carcinomas. Histological investigations proved a continuous development of the new tumors from the transplanted tumor cells, and no indication was found which would support the assumption that an agent was liberated from the inoculated cells and led, in turn, to a malignant transformation of normal cells of the host.[256]

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[241] E. Wollman, M. A. Macheboeuf, M. Bardach & J. Basset: Compt. rend. Soc. de biol. *123*: 588 (1936).

[242] J. Basset, E. Wollman, M. A. Macheboeuf & M. Bardach: Compt. rend. Acad. d. sc. *200*: 1247 (1935).

[243] J. Basset, M. Macheboeuf & E. Wollman: Ann. Inst. Pasteur *58*: 58 (1937).

[244] J. Furth, A. Tuggle & C. Breedis: Proc. Soc. Exper. Biol. & Med. *38*: 490 (1938).

[245] L. Heidenhain: Über das Problem der bösartigen Geschwülste, Springer 1928; Ztschr. f. Krebsforsch. *26*: 492 (1928).

[246] J. V. Andersen: Über Karzinome, Sarkome und Lymphomatosis infiltrans bei weissen Mäusen, Levin & Munksgaard, Copenhagen 1934.

[247] G. Klein: Arch. f. klin. Chir. *183*: 194 (1935).

[248] R. Meyer: Klin. Wchnschr. *14*: 971 (1935).

[249] L. Aschoff: Libro de Oro, Buenos Aires, p. 47 (1935).

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[250] J. Klinke: Arch. f. klin. Chir. *188*: 591 (1937).

[251] H. Auler & B. Hohenadel: Ztschr. f. Krebsforsch. *48*: 149 (1938).

[252] F. Gerlach: Wien. klin. Wchnschr. *50*: 1180; 1603 (1937).

[253] L. D. Parsons: J. Path. & Bact. *43*: 11 (1936).

[254] U. Kreide & H. Kudicke: Frankfurt. Ztschr. f. Path. *52*: 407 (1938).

Notwithstanding these numerous objections and the lack of direct proof for the general induction of malignant tumors by a virus, or viruses, a number of authors maintained the probability of a virus infection being responsible for neoplasia in general, and enumerated all factors which appeared in favor of this theory.[174, 257–259] In particular, it has been stated that viruses represent a "proximate cancer-cause," while all other carcinogenic factors are to be considered "remote cancer-causes" which make the tissue susceptible to the virus action and consequent malignant transformation.[260] Similarly, the theory has been advanced that a virus may produce malignant growth during the healing process of chronic infections and that pre-cancerous lesions, when contaminated with the specific tumor virus, will result in neoplastic development. It was stressed that the resistance of the body is of great importance in the event of an infection by the hypothetical tumor virus, and an analogy with other infectious diseases of known nature was pointed out: "—we

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255. J. Flaks: Bull. Assoc. franç. p. l'étude du cancer *25*: 411 (1936).
256. J. Dischreit: Frankfurt. Ztschr. f. Path. *49*: 489 (1936).
257. W. E. Gye: Lancet *2*: 199 (1926); 2nd Internat. Cancer Congress, Brussels *1*: 59 (1936).
258. W. E. Gye & W. J. Purdy: The Cause of Cancer, Cassel & Co., London 1931.
259. C. H. Andrewes: Lancet *2*: 63; 117 (1934).
260. W. E. Gye: Brit. M. J. *1*: 551 (1938).

are probably all exposed to cancer as we are all exposed to poliomyelitis. Many perhaps develop small cancerous lesions which heal without their presence even being known, as happens in the case of tuberculosis."[261] As has been suggested in the introduction to this survey, these theories which regard a virus as the causative agent of neoplasia in general have by no means been definitely proven; yet, in spite of several findings already discussed, which apparently lessened the probability of such assumptions, they cannot be, at present, unconditionally rejected. A final decision of this problem could be made only in one of two events; an answer in the affirmative would require the demonstration of a virus, or of viruses, in all types of malignant tumors and experimental evidence that these agents are capable of eliciting neoplasia regularly. On the other hand, a complete rebuttal of the virus theory would be rendered possible by the establishment of another theory capable of explaining the theoretical and experimental aspects of cancer etiology to full satisfaction. Yet, in connection with the virus theory of cancer origin, one circumstance shall be pointed out which becomes apparent by taking into consideration the established facts of carcinogenesis induced by means of defined chemical or physical stimuli. When the virus theory implies that these forms of neoplasia are due in reality not to the carcinogens employed, but to a virus which acts upon the chemically or physically "prepared" tissue, then, in view of the regularity with which these carcinogens induce cancer, it must be concluded that the alleged virus is ubiquitous and, in this event, its role in cancer causation would have to be regarded as rather subordinate, since its activity would depend wholly upon the preceding "preparatory action" of the carcinogenic stimuli of known—or unknown—nature.

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261. D. T. Quigley: Am. J. Roentgenol. *44*: 110 (1940).

CHAPTER X

CHEMICAL AND BIOLOGICAL TUMOR DIAGNOSTICS

THERE is no doubt in the authors' mind that in this concluding chapter the perhaps most delicate and most hotly disputed field of cancer research is entered into. The reason for this may be seen in the fact that this branch of oncology is not merely concerned with a theoretical elaboration of scientific problems, but aims primarily at immediate practical results. Accordingly, the work on biological and chemical cancer diagnosis differs from that devoted to other aspects of the tumor problem, in that its results are subject, so to speak, to the law of "all or nothing"; the final evaluation of a proposed method entails a complete rejection, if re-examinations and statistical analyses reveal that the test in question is not able to reach the goal set, i.e., results of a reliability and an accuracy justifying the clinician using them as a safe guide for his therapeutic actions. It is obvious that such decisions cannot be made lightly since the life of a patient may depend on them. Therefore, it is quite comprehensible that attempts to secure such means of cancer diagnosis, which go back almost half a century, have given rise to a great many disappointments because announcements of positive achievements in this field, forthcoming from various authors, have repeatedly been found invalidated in the light of reinvestigations performed by other workers. Naturally such sharp conflicts evoked a strong scepticism as to the possibility of attaining the desired aim not only by means of the tests under present discussion, but also as to other methods which may be developed in the future.

One of the factors responsible for the attitude of many authoritative oncologists, who believe that the past record has completely discredited endeavors to create specific methods of cancer diagnostics, is that all too frequently, deviations from the normal observed in the course of neoplastic diseases, have been proclaimed as warranting a diagnostic utilization before any evidence had been furnished which demonstrates the phenomena in question as being primarily connected with the tumor process. It will be recalled that in the preceding chapters a great many instances of this kind were enumerated when attacks on the cancer problem from most varied angles —inorganic, organic, or physical chemistry, metabolic or endocrine mechanisms—have been undertaken. The results obtained have been searched under the pressure of dire necessity for keys which might unlock the mysterious gate barring entrance to successful tumor diagnostics which might be performed by other means than those known at present, i.e., of morphological or radiological nature. In this chapter, with few exceptions, the futile efforts previously cited will not again be mentioned, but the survey will be generally limited to investigations undertaken on the basis of experimental evidence or of theoretical premises, which at least offer some likelihood for the accomplishment of useful results. No detailed account of the technical performance of the individual tests can be given, such an account being outside the scope of this book, but emphasis will be laid on their theoretical backgrounds, which may prove of interest and value even in instances where the practical results must be considered as failures. At the same time, these theoretical foundations, it is hoped, will furnish viewpoints for a lucid arrangement of the vast material recorded; this will be divided mainly into sections dealing with immunological, enzymatic or colloid-chemical tests, or into those in which determinations of chemical or physicochemical constants have been utilized. The great majority of the methods to be discussed have employed blood serum or blood as substrates, while other easily accessible substrates, such as urine, feces, etc., have been used to a lesser extent. Some diagnostic tests proposed represent biological reactions of the whole organism, and in such instances phenomena are involved which at present are not clearly defined in biochemical terms. However, since all borderlines in natural sciences are more or less arbitrary, and such delimitations are subject to frequent revision in the course of advancing knowledge, these tests will also be included in the following presentation, because future work may reveal their dependence on chemical factors.

1. *Immunological Reactions.*

This group of diagnostic reactions is considered first, since it represents a trend of work which has scored many successes in the diagnosis of numerous infectious diseases, and accordingly, similar methods were soon introduced into cancer research. Yet, in this respect, the hopes placed in the effectiveness of the immunological approach have not been fulfilled, although up to the most recent times efforts have been made to reconsider this problem, and to effect its solution. But, as has been stated in chapter VIII, the fundamental question has not as yet been answered unequivocally, whether or not antigens and antibodies, specific for neoplasms, may be demonstrated to exist, and hence the basic premise for an immunological cancer diagnosis does not appear to be established. In accordance with this theoretical difficulty, none of the numerous immunological tests devised has been found of definite practical value.

Complement-fixation tests. This classic immunological reaction, discovered by Bordet-Gengou, has attracted the attention of cancer research workers, on account of its highly satisfactory results, in the Wassermann reaction, for the serodiagnosis of lues. A number of authors applied modifications of such a fixation of complement to cancer diagnosis by using aqueous or alcoholic tumor antigens,[1-4] or even such unspecific antigens as phenolphthaleine or maltose,[5] but no clinically useful results have been obtained. In extensive studies, alcoholic extracts of tumors, and also of red blood cells, were prepared by special methods and served as antigens; at least in advanced cancer cases, this method yielded satisfactory results,[6] but further experiences with this test [7-9] did not sustain its clinical practicability, since correct results appeared in 75 to 90 per cent of cancer patients, but erroneous diagnoses of 7 to 30 per cent were found in control cases.[8, 9] In the course of studies on the meiostagmin reaction, which will be discussed later, it has been observed that interaction of methyl alcoholic tumor extracts and of tumor sera led to formation of precipitates which bound complement,[10] but only a few experiences were collected with this reaction.

It has become obvious that the failure of this older work cannot be charged wholly to insufficient theoretical knowledge or to technical imperfections in the field of serology, since the

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[1] G. Sampietro & D. Tesa: Ann. d'ig. sper. *18*: 657 (1908).

[2] C. E. Simon & W. S. Thomas: J. Exper. Med. *10*: 673 (1908).

[3] Weinberg & U. Mello: Bull. Assoc. franç. p. l'étude du cancer *3*: 43 (1910).

[4] C. S. Engel: Ztschr. f. Krebsforsch. *10*: 248 (1911).

[5] K. Hara: Deutsche med. Wchnschr. *40*: 484 (1914).

[6] E. v. Dungern: Deutsche med. Wchnschr. *38*: 1215 (1912); München. med. Wchnschr. *59*: 238; 1093; 2834 (1912).

[7] D. Edzard: Berlin. klin. Wchnschr. *49*: 2488 (1912).

[8] F. Schenk: Wien. klin. Wchnschr. *26*: 529 (1913).

[9] J. Halpern: München. med. Wchnschr. *60*: 914 (1913).

[10] G. Izar; quot. M. Ascoli: Ergebn. d. inn. Med. u. Kinderh. *25*: 944 (1923).

application of the most modern methods and experience likewise did not accomplish essentially better results.[11-13] Theoretical aspects stood in the foreground of investigations in which immune sera were produced in animals by injecting them with boiled cancer tissue, and these sera were tested for complement-fixation using cancer lipids as antigens.[14] Extensive clinical studies have been performed by a group of authors who also used tumor lipids as antigens for complement-fixation with sera of cancer patients. Here, only fifty to sixty per cent of correct results were found by applying this method to the sera of patients suffering from cancers of internal organs, while cancers of other localizations yielded still less favorable results.[15-17] Sera of cancer-free persons in general did not show complement-fixation with this antigen, excepting those affected with lues, which disease is detected by the Wassermann reaction performed simultaneously;[18] on the other hand, some cancer sera reacted unspecifically with lipids extracted from normal organs.[19] Hence, the authors themselves interpreted their findings as excluding a clinical value for this test, which interpretation is in agree-

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11. J. A. B. Hicks & F. M. D. Hocking: Internat. Conf. Cancer, London, p. 447 (1928).

12. L. H. Peretz & A. J. Newler: Ztschr. f. Krebsforsch. *41*: 267 (1935).

13. J. Putnoky: Frankfurt. Ztschr. f. Path. *48*: 335 (1935).

14. H. Lehmann-Facius: Klin. Wchnschr. *9*: 2430 (1930); Ztschr. f. Immunitätsforsch. u. exper. Ther. *67*: 373 (1930).

15. L. Hirszfeld, W. Halber & J. Laskowski: Ztschr. f. Immunitätsforsch. u. exper. Ther. *64*: 61; 80 (1929).

16. L. Hirszfeld: Klin. Wchnschr. *8*: 1365 (1929); 2nd Internat. Cancer Congress, Brussels *1*: 227 (1936).

17. L. Hirszfeld, W. Halber, M. Floksztrumpf & J. Kolodziejski: Ztschr. f. Immunitätsforsch. u. exper. Ther. *67*: 286 (1930); Bull. internat. Acad. pol. sc., Cl. méd. No. 6/7 (1934); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 164 (1935).

18. M. Floksztrumpf & J. Kolodziejski: Klin. Wchnschr. *10*: 1120 (1931).

19. L. Hirszfeld, W. Halber & Z. Szwojnicka: Klin. Wchnschr. *14*: 154 (1935).

ment with other reports employing the same technique.[20, 21] Furthermore, a detailed analysis demostrated the impracticability of this reaction, since only 17.5 per cent of 204 cancer sera, and 9.5 per cent of control sera yielded positive results,[22] and antigens derived from normal organs reacted similarly to those prepared from tumors.[23] Lipid antigens have been recently isolated from mouse sarcomas 37, from tar cancers of mice, and also from mouse tumors induced by methylcholanthrene, and complement-fixation was demonstrable when the lipids were brought together with sera of the tumor-bearing animals named, while normal mouse tissue was neither found to contain a corresponding antigen, nor did normal mouse sera contain corresponding antibodies.[24] These observations indicate, perhaps, that in tumor-bearing animals simpler immunological relations prevail than in humans afflicted with cancer. A theory which saw the cause of cancer in a myxomycetes infection led to the elaboration of a complement-fixation test by using an antigen prepared from these microorganisms, and excellent results were claimed to have been obtained.[25] Since the "cancer theory" mentioned is generally rejected, and since no re-examinations of the test connected with it are available, scientific value can not be assigned to this claim.

Precipitin tests. The mechanism of these reactions is closely related to the complement-fixation, since present-day opinions regard the precipitation as a visible expression of the binding between antigen and antibody. Accordingly, the serodiagnosis of cancer made with these methods exhibits

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[20] F. P. Tinozzi: Diag. e tec. di lab. *3*: 8 (1932).
[21] W. Saphir & N. Hirschberg: J. Immunol. *25*: 439 (1933).
[22] A. Zacho: Ztschr. f. Krebsforsch. *43*: 434; *44*: 43; 79; 353; 359 (1936).
[23] A. Zacho & E. Poulsen: Ztschr. f. Krebsforsch. *43*: 458 (1936).
[24] L. Hoyle: Am. J. Cancer *39*: 224 (1940).
[25] W. Schmidt: Ztschr. f. Krebsforsch. *28*: 545 (1927).

essentially the same aspects as the aforementioned complement-fixation tests. Sera of rabbits which had been treated with injections of cancer sera appeared to contain a specific precipitin, as demonstrated in a flocculation occurring when these sera were mixed with sera of cancer patients.[4] Somewhat different theoretical premises formed the basis for a precipitin test in which a specific turbidity was claimed to result from the action of cancer sera on aqueous tumor extracts; [26] the presence of an unsaturated dicarboxylic acid in the sera of cancer patients was held responsible for this phenomenon, which has been confirmed by some older work ([27, 28,] cf. carcinolytic reaction). Another precepitin test goes back to the meiostagmin reaction; an emulsion of tumor antigen, mixed with the serum to be tested and incubated for twelve hours, was stated to show the formation of a fine precipitate and a clearing of the emulsion, when the serum was derived from a cancer patient. Positive results were seen in eighty-three per cent of tumor patients, but also in fourteen per cent of cancer-free persons.[29] Similar findings were obtained by using emulsions of ricinoleic or linoleic acid,[10, 30, 31] or an emulsion of oleic acid as antigens.[32] Particular interest may be ascribed to those methods in which lipid extracts prepared from neoplasms were employed; among these may be placed the Shaw-Mackenzie test,[33] according to which the stratifying of cancer serum with a fatty acid extract of tumors should result in a specific turbidity following an incubation of eighteen hours; however,

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26. E. Freund & G. Kaminer: Biochem. Ztschr. *46*: 470 (1912).
27. L. Arzt & W. Kerl: Wien. klin. Wchnschr. *25*: 1821 (1912).
28. U. Mello: Compt. rend. Soc. de biol. *74*: 231 (1913).
29. A. Stammler: München. med. Wchnschr. *58*: 1957 (1911).
30. G. Izar: Klin. Wchnschr. *2*: 641 (1923).
31. M. Ascoli & G. d'Alessandro: Biochim. e terap. sper. *20*: 94; 518 (1933).
32. H. Kahn: Klin. Wchnschr. *2*: 128 (1923).
33. J. A. Shaw-Mackenzie: Lancet *2*: 759 (1922).

several re-investigations failed to corroborate this claim.[34, 35] Another flocculation test has been tried by Fry;[36] this author obtained seventy-five per cent positive results with cancer sera mixed with alcoholic carcinoma extracts, but observed unspecific reactions with sera of luetic or tuberculous patients, and here again later studies did not demonstrate a usefulness of this procedure.[37, 38] Similarly, a precipitation test in which a protein antigen is used[39] has not been found of definite value. In analogy to the Wassermann reaction, where the originally used extracts of syphilitic organs were later replaced by antigens derived from normal tissues, attempts have been made to employ unspecific antigens for the cancer diagnosis, but much less satisfactory accomplishments have been recorded than in the case of lues diagnosis. Thus, alcoholic beef heart extracts were used for a precipitin test, leading to ninety-eight per cent negative results in cancer-free persons and to seventy-nine per cent positive reactions in tumor patients,[40] but a re-examination yielded much less favorable findings.[41] Likewise, in another study, alcoholic tumor or beef heart extracts were employed with similar results; the authors found that the addition of electrolytes, particularly of calcium acetate, improved the effectiveness of the methods which were applied to approximately 1000 sera, but correct results were obtained only in eighty per cent of cancer patients

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[34] E. C. Dodds & W. Lawson: Internat. Conf. Cancer, London, p. 443 (1928).

[35] E. Watchorn: J. Hyg. *29*: 71 (1929).

[36] H. J. B. Fry: Brit. M. J. *2*: 4 (1925); J. Path. & Bact. *29*: 353 (1926).

[37] C. Cerruti & L. Biançalana: Minerva med. *7*: 816 (1927); abstr. Cancer Rev. *3*: 370 (1928).

[38] P. Piotrowski: Bull. Assoc. franç. p. l'étude du cancer *16*: 56 (1927.)

[39] B. Gruskin: Am. J. M. Sc. *177*: 476 (1929).

[40] E. Weiss: Arch. Path. *13*: 106 (1932).

[41] R. A. Kilduffe: Arch. Path. *15*: 393 (1933).

and in ninety per cent of control cases.[42] It is noteworthy that eighty per cent of correct findings in cancer patients appears to represent the limit of the effectiveness of most of the tests given, thus practically excluding their clinical usefulness. Furthermore, some doubt must be expressed as to whether immunological factors are responsible for the phenomena observed, or whether they are not rather caused by physicochemical alterations of the sera analyzed (cf. sec. 3). Such an involvement of colloidchemical changes in the cancer serum may be assumed with great probability as causing flocculations in cancer sera after the addition of oleate solutions.[32, 43] More specific factors have been believed to participate in a reaction which was developed by Lehmann-Facius,[44] that is, a flocculation test in which phospholipids, prepared from carcinomas by special methods, serve as antigens, and the antibodies would be represented by the euglobulin fraction of the serum of cancer patients. Whereas the author reported a comparatively high specificity of this method, a re-investigation arrived at considerably less favorable results.[45] Later, Lehmann-Facius [46] proposed modifications of his test, which might improve its effectiveness; lecithin was added to the antigen, prepared from Brown-Pearce rabbit carcinoma; the sera tested were inactivated, thus destroying unspecific thermolabile factors, while the specific antibodies of the cancer serum were claimed to be thermo-

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42. M. L. Chevrel-Bodin, F. Chevrel & M. Cormier; Ann. Inst. Pasteur *55*: 108 (1935); Compt. rend. Soc. de biol. *118*: 520 (1935).

43. H. Kahn: Klin. Wchnschr. *2*: 1364; 2241 (1923); *3*: 920 (1924); Strahlentherapie *15*: 808 (1923/24).

44. H. Lehmann-Facius: Klin. Wchnschr. *11*: 775 (1932).

45. H. W. Lundy: J. Lab. & Clin. Med. *20*: 923 (1935).

46. H. Lehmann-Facius: Klin. Wchnschr. *15*: 1591 (1936); Deutsche med. Wchnschr. *63*: 1274 (1937); in C. Adams & H. Auler: Neuere Ergebnisse auf dem Gebiete der Krebskrankheit, S. Hirzel, Leipzig 1937, p. 129.

stable. Extensive work with a modification of this test has also been undertaken by Nakagawa and his collaborators,[47-52] and very good results were reported by them on the basis of a large amount of material analyzed. But in contrast to these claims this modification yielded only eighty per cent correct results in the hands of another author who, therefore, doubted its specific character.[53] A high percentage of unspecific reactions (up to fifty per cent) with sera of cancer-free persons was also obtained in re-investigations of the modified Lehmann-Facius reaction, whereas the correct results in cancer patients ranged between seventy and eighty per cent.[54-56] In other precipitin tests, lipid antigens were utilized, extracted from tumors,[57] or from blood,[58] but their usefulness cannot be evaluated at present, since these methods have not undergone testing on a broader scale. This is also the case regarding a somewhat different technique, viz., a cold-flocculation. For this purpose alcoholic extracts of human or animal tumors were used, and when these antigens were admixed with sera of cancer patients and exposed to low

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47. S. Nakagawa, T. Takasugi, S. Ogawa & J. Yoshida: Klin. Wchnschr. *13*: 1775 (1934).
48. S. Nakagawa, T. Takasugi & J. Yoshida: Klin. Wchnschr. *14*: 1218 (1935).
49. S. Nakagawa, T. Takasugi & M. Sato: Klin. Wchnschr. *15*: 21 (1936).
50. S. Nakagawa, T. Takasugi & S. Ogawa: Klin. Wchnschr. *15*: 1204 (1936); Ztschr. f. Krebsforsch. *44*: 455 (1936).
51. S. Nakagawa: Deutsche med. Wchnschr. *63*: 1275 (1936); Ztschr. f. Krebsforsch. *45*: 488 (1937).
52. S. Nakagawa, S. Ogawa & K. Kurosawa: Ztschr. f. Krebsforsch. *46*: 403 (1937).
53. C. Aoki: Gann *32*: 485 (1939).
54. F. Chrometzka & W. J. Schulte: Deutsche med. Wchnschr.. *62*: 1952; *63*: 1274 (1937).
55. W. Geisthövel: Zentralbl. f. Chir. *65*: 2642 (1938).
56. D. Albers: Deutsche med. Wchnschr. *65*: 1083 (1939).
57. J. L. Landau & W. M. German: Am. J. Clin. Path. *2*: 343 (1932).
58. R. Ibáñez: Arch. españ. oncol. *3*: 245 (1933); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 164 (1935).

temperature, considerable flocculation was observed, whereas sera of cancer-free patients did not react in this manner. Sera of rabbits, which had been treated with tumor tissue, exhibited a behavior similar to that of cancer sera.[59] In summarizing, it may be inferred that the application of precipitin tests has thus far not developed to a point where their clinical use for diagnosis of neoplasia would be justified, and hence only theoretical interest can be ascribed to the statement that flocculation reactions are more suitable for the demonstration of tumor-specific immune bodies than methods based on fixation of complement.[60]

In conjunction with precipitins, an agglutination test may be mentioned, according to which sera of cancer patients cause a specific agglutination of Coli bacteria which have been cultivated from the intestinal contents of cancer patients.[61] The theoretical foundation of this test goes back to the conceptions of Freund and Kaminer, which will be described in detail later.

Epiphanin reaction. An interesting criterion for the recognition of an ensuing binding between antigen and antibody has been established in the form of the so-called epiphanin reaction. This is based on the observation that the neutralization points of solutions of barium hydroxide and of sulfuric acid are markedly shifted, in the event of antigen-antibody reactions occurring.[62] This phenomenon, believed to be generally applicable for immunological purposes, has also been employed for tumor diagnosis, by using a tumor extract as antigen, and the results obtained corresponded approximately to those of the other methods quoted above.[63]

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59. K. Pelczar: 1st Internat. Cancer Congress, Madrid *1*: 209 (1933).
60. O. Sievers: Ztschr. f. Krebsforsch. *41*: 307 (1935).
61. G. Kaminer: Wien. klin. Wchnschr. *48*: 508 (1935).
62. W. Weichardt: Ztschr. f. Immunitätsforsch. u. exper. Ther. *6*: 644 (1910).
63. E. Jósza & M. Tokeoka: Deutsche med. Wchnschr. *40*: 590 (1914).

Lysins. In the following the demonstration of hemolysins and of their inhibitions will alone be surveyed, while the studies dealing with carcinolytic (cytolytic) phenomena are reserved for a separate presentation. Furthermore, it must be remarked that the immunological nature of some observations on hemolysis, which are to be discussed, is very questionable, and the involvement of unspecific physicochemical factors very probable.

Isolysins. In accord with other reports on the presence of isolysins in the sera of tumor patients,[64, 65] demonstrations have been made on a broader scale which showed that eighty-three per cent of tested cancer sera were capable of dissolving red blood cells of normal individuals.[66] It was noted, however, that among the negatively reacting sera were also those of some persons afflicted with far-advanced neoplasms, and that, on the other hand, positive reactions were obtainable in ninety-two per cent of sera of tuberculosis patients. Several later studies led to analogous results,[67, 68] and the conclusion was drawn that only a negative outcome of the test might be useful in practice, insofar as malignancy could thus be excluded.[69] Unspecific reactions were also observed in some acute infections and in severe anemias,[70] factors, which —together with the not quite simple technique of the test— precluded its continued application.

Heterolysins. The only contribution made to this field bears a highly problematic character, since it was derived from the very peculiar theory that malignant tumors allegedly contain proteins foreign to the species. At any rate, it was

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64. L. Bard: Compt. rend. Soc. de biol. *53*: 170 (1901).
65. M. Ascoli: München. med. Wchnschr. *48*: 1239 (1910).
66. G. W. Crile: Ann. Surg. *47*: 1063 (1908).
67. B. Agazzi: Berlin. klin. Wchnschr. *47*: 1454 (1910).
68. H. Upcott: Lancet *2*: 720 (1910).
69. O. P. Johnstone & C. H. Canning: J. A. M. A. *53*: 1479 (1910).
70. H. L. Richartz: Deutsche med. Wchnschr. *35*: 1340 (1909).

claimed that the majority of sera of cancer patients exhibited an increased hemolytic power towards animal erythrocytes (of fowl, sheep, beef, pig); [71] however, not only theoretical reasons, but also practical re-examination of the test, led to its rejection.[72, 73]

Inhibition of hemolysis. It has been observed that the hemolytic activity of myristylic acid, of linoleic acid or of ricinoleic acid, was reduced more markedly by the addition of sera of tumor-free persons, than by the sera of cancer patients.[40] Analogous findings were obtained regarding hemolysis by means of pancreas extracts [74] or of bile.[75] However, all these tests were not considered as being specific enough to warrant their recommendation for clinical use.[76] As stated above, the phenomena of hemolysis inhibitions most likely are determined by physicochemical factors, and, in particular, the observation that tumor-sera inhibit hemolysis, caused by sodium oleate, to a lesser extent than normal sera,[77, 78] has been thought to depend on a diminution of the albumin-A fraction in the sera of cancer patients,[77] and for this reason this test was abandoned in favor of albumin-A determinations (cf. p. 853).

Hemolysis of erythrocytes of tumor patients. Reports on an anomalous behavior of red blood cells in neoplastic diseases have been mentioned in chapters II and III and may be briefly repeated here. The erythrocytes of patients with sarcoma were found to be less resistant against the hemolytic action of snake venom than normal ones, whereas the red blood cells of carcinoma-bearers showed an increased resistance, but these findings were not constant enough for diagnostic purposes.[79, 80] Likewise, it seems to be more of theoretical interest than of practical value that erythrocytes of tumor patients exhibited a greater sensitivity toward several photodynamic substances (fluorescein, erythrosin, chlorophyll) than blood cells of normal persons.[81]

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[71] G. Kelling: Berlin. klin. Wchnschr. *42*: 911; 950 (1905); *44*: 1293 (1907); Wien. klin. Wchnschr. *19*: 585 (1906); *21*: 1292 (1909).

[72] E. v. Dungern: Ztschr. f. Krebsforsch. *5*: 48 (1907).

[73] E. Fuld: Berlin. klin. Wchnschr. *42*: 535; 955 (1905).

[74] N. Waterman: Nederl. tijdschr. v. geneesk. *2*: 2176 (1915).

[75] H. A. Dietrich: Ztschr. f. Geburtsh. u. Gynäk. *81*: 641 (1918); Klin. Wchnschr. *1*: 2382 (1922).

[76] G. Izar: Klin. Wchnschr. *1*: 1695 (1922).

[77] H. Kahn & P. Potthof: Ztschr. f. d. ges. exper. Med. *31*: 423 (1923).

[78] H. Decker: Ztschr. f. d. ges. exper. Med. *38*: 174 (1923).

Skin tests. The outstanding success of cutaneous tuberculin diagnostics and favorable results accomplished by means of skin tests in various infectious diseases led to experiments utilizing similar phenomena for tumor diagnosis. Satisfactory results have been described in a study in which intracutaneous injections of glycerine extracts of mammary carcinoma were used.[82] On the recommendation of Abderhalden, attempts were made to employ for skin tests the sera of horses which had been immunized with tumor tissue. This method yielded eighty-six per cent of correct results in patients with adenocarcinomas, but numerous unspecific reactions were also observed.[83, 84] In addition, it has been asserted that the intradermal injection of roentgen-irradiated serum of cancer patients evokes a specific skin response,[85] and one investigator with this method obtained twenty-three positive reactions among twenty-six cancer patients,[86] but no further analysis

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79. R. Kraus, O. Pötzl, E. Ranzi & H. Ehrlich: Wien. klin. Wchnschr. *22*: 1027 (1909).

80. R. Kraus, E. v. Graff & E. Ranzi: Wien. klin. Wchnschr. *24*: 1003 (1911).

81. A. H. Roffo: Bol. inst. med. exper. para el estud. y trat. d. cáncer *7*: 595 (1932); Ztschr. f. Krebsforsch. *38*: 312 (1932).

82. C. Achard, H. Berinard & C. Gagneux: Bull. Assoc. franç. p. l'étude du cancer *3*: 66; 209 (1910).

83. O. Boyksen: München. med. Wchnschr. *66*: 93 (1919); Ztschr. f. Krebsforsch. *23*: 110 (1926).

84. R. Wigand: Ztschr. f. Immunitätsforsch. u. exper. Ther. *36*: 202 (1923).

85. V. E. Mertens: Deutsche Ztschr. f. Chir. *179*: 216 (1923).

86. F. Hoff & K. Schwarz: München. med. Wchnschr. *71*: 816 (1924).

of this test has been recorded. In the skin test of Freund and Kaminer,[87] an unsaturated dicarboxylic acid, isolated from the intestinal contents of cancer patients, is injected intradermally, and formation of characteristic wheals was claimed to occur in cancer patients, but not in cancer-free persons. The authors reported on 96.5 per cent correct results in 261 tested cancer cases, and on 84.8 per cent correct results in 176 control cases. Numerous authors have published their experiences as collected with this method,[88-94] but it must be noted that the amount of erroneous diagnoses in cancer-free persons—averaging in these investigations twenty per cent—must be considered as definitely impairing the clinical usefulness of the test. In this respect, moreover, reference is made to other studies according to which unspecific reactions were even found in thirty to fifty per cent of control cases,[95-97] a fact which obviously precludes the possibility of a practical application of the method in question. Intracutaneous injections of Berkefeld filtrates of tumor tissue have been employed without much success,[98] somewhat better results were seen with a skin test using albumin solutions prepared from the sera of cancer patients.[59] Another skin test has been based on a concept, similar to a theory mentioned above, according to which malignant tumors contain proteins foreign to the species, and it was claimed that intradermal injection of protein antigen derived from calf or pig embryos resulted in skin reactions specific for carcinoma, or sarcoma, respectively.[99] However, apart from the questionable evidence presented for the theoretical premise, the test has been found valueless in actual re-examination.[100] Unspecific antigens have been employed in some skin tests proposed for cancer diagnosis. In older studies, emulsions of red blood cells, derived from normal humans, were injected intracutaneously and a characteristic skin reaction was seen in one hundred per cent of cancer patients, but only in three per cent of cancer-free cases;[101, 102] another study, however, failed to confirm this claim.[103] More recently, intracutaneous injection of hydatid liquid (echinococcus test) was described as being a promising means of cancer diagnosis,[104] but no further experiences with this method have been reported.

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[87] E. Freund & G. Kaminer: Sitzg. d. Gesellsch. d. Ärzte in Wien, Jan. 30, 1931; Wien. klin. Wchnschr. *46*: 1576 (1933).

[88] G. Marangos: Wien. klin. Wchnschr. *45*: 848 (1932).

[89] M. W. Mettenleiter: Wien. klin. Wchnschr. *47*: 1519 (1934); Monatschr. f. Krebsbekämpf. *3*: 240 (1935).

[90] V. Orator & Arens: Wien. med. Wchnschr. *84*: 260 (1934).

[91] J. Cholewa & S. Černelc: Wien. klin. Wchnschr. *46*: 1072 (1933).

[92] A. E. Klein: Wien. klin. Wchnschr. *46*: 1586 (1933).

[93] K. Pichler: Klin. Wchnschr. *13*: 451 (1934).

[94] J. Cholewa: Libro de Oro, Buenos Aires, p. 217 (1935).

[95] H. Kotrnetz & H. Weber: Deutsche Ztschr. f. Chir. *240*: 533 (1933).

[96] R. E. Buchwald: Zentralbl. f. Gynäk. *62*: 60 (1938).

[97] H. Dietel: Geburtsh. u. Frauenheilk. *2*: 99 (1940).

[98] M. Cutler & W. Saphir: J. Allergy *4*: 389 (1933).

Miscellaneous immunological tests. In contrast to the methods discussed so far, in which—with the exception of the skin tests—laboratory procedures were employed for cancer diagnosis, a number of experiments have been aimed at evoking general reactions in cancer-afflicted patients, which might facilitate a differentiation of this disease from other pathologies. A large amount of work has dealt with the "hemoclastic crisis" i.e., a leucocyte drop, similar to Widal's phenomenon, which was claimed to occur in cancer patients thirty minutes after the injection of tumor extracts prepared by special methods.[105] Although the authors of this test recorded ninety-one per cent correct results in tumor patients, and ninety-four per cent in control cases, and similar findings were published in several communications,[106-112] other reexaminations led to less favorable results and caused doubt as to a practical value of the test,[113-116] a view which seems confirmed by more recent work in which erroneous results were found in about twenty per cent of both cancer and control cases.[117] Incidentally, it was also reported that an alimentary leucopenia, in the sense of Widal, occurred in about eighty per cent of cancer patients; but no specific nature was assigned to this observation.[118] Anaphylactic reactions have been described as following injections of tumor extracts to cancer patients,[119] but the insufficient elaboration of this finding does not permit a definite evaluation.

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[99] B. Gruskin: J. Lab. & Clin. Med. *17*: 1237 (1932).

[100] J. McFarland, J. H. Clark & M. Friedmann: J. Lab. & Clin. Med. *20*: 468 (1935).

[101] C. A. Elsberg: J. A. M. A. *52*: 1036 (1909).

[102] C. A. Elsberg, H. Neuhof & S. H. Geist: Am. J. M. Sc. *139*: 264 (1910).

[103] G. Wolfsohn: Berlin. klin. Wchnschr. *47*: 1995 (1910).

[104] V. S. Perpiñá & F. S. de Frutos: Zentralbl. f. Chir. *59*: 2408 (1932).

[105] M. A. Piazza: Minerva med. *1*: 165 (1930).

A different effect of general biological nature is exhibited by the Moppett reaction in which cultures of neoplastic tissue are used as test objects. The principle of this method is based on the observation that such tissue cultures, when suitably placed in capillaries between the blood of normal persons and of cancer patients, show a characteristic growth pattern; that

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106. P. Carco: Arch. pat. e clin. med. *10*: 98 (1930); abstr. Cancer Rev. *6*: 490 (1931).
107. E. Giuffrida: Minerva med. *1*: 525 (1932).
108. S. Barbera: Oto-rino-laring. ital. *2*: 132 (1932).
109. G. Zanni: abstr. Ztschr. f. Krebsforsch. *41*: Ref. 26 (1935).
110. A. Longo: Arch. di. ostet. *37*: 1 (1930); abstr. Cancer Rev. *6*: 27 (1931).
111. G. Bossa: Policlinico (sez. med.) *38*: 82 (1931).
112. P. Restivo (1931); abstr. Cancer Rev. *7*: 386 (1932).
113. P. Natale: Ann. ostet. *53*: 2169 (1931); abstr. Ztschr. f. Krebsforsch. *38*: Ref. 24 (1932).
114. R. Bolaffi: Riv. ital. ginec. *13*: 172 (1932); abstr. Ztschr. f. Krebsforsch. *38*: Ref. 24 (1932).
115. G. Rotolo: Atti e mem. soc. lomb. chir. *2*: 603 (1934); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 75 (1935).
116. E. Gay: Riv. di clin. med. *33*: 671 (1932).
117. G. B. Fornari: Bol. soc. med.-chir. Catania *4*: 614 (1936); abstr. Ztschr. f. Krebsforsch. *45*: Ref. 345 (1937).
118. K. Rausche: München. med. Wchnschr. *73*: 441 (1926).
119. S. Bojovic: Srpski Arch. Lekarst. *36*: 449 (1934).

is, the cancer blood "repels" the emigration of cells from the neoplastic tissue. In this experiment, substituting the cancer blood by the sample to be tested, it should be possible to decide whether it was derived from a normal or a tumor-bearing individual, and the author asserted a diagnostic usefulness existed for this procedure.[120] In one re-examination, however, one hundred cases were tested in this manner, and while correct results were registered in ninety per cent of control cases, only sixty-two per cent positive results were found with the blood of cancer patients.[121] Considering this result and the technical difficulties of the test, its practical application does not seem too encouraging.

2. *Enzymatic Reactions.*

The tests which are discussed in this section to a great extent are not dependent on enzymatic processes common to normal tissues and body fluids, but represent phenomena of enzymatic action which are believed to be specific for neoplastic conditions. This applies particularly to .the Abderhalden test, to the carcinolytic test (Freund and Kaminer) and to the fibrinolytic test (Fuchs), but, in addition, several diagnostic methods, which are based on alterations of physiological enzymes, supposedly taking place in malignancy, will be surveyed, and these were in part enumerated in chapter IV.

Abderhalden test. Abderhalden [122] initiated a trend in experimental work which has been strongly influenced by immunological concepts, inasmuch as he started from the premise that parenteral administration of complex, chemically defined substances (proteins, polysaccharides, fats) may result in the production of new enzymes capable of effecting a specific cleavage of the employed "antigens." The presence of these enzymatic factors, termed "defensive ferments," could be demonstrated in the blood of experimental animals, i.e., after parenteral injections of lactose a lactase was formed; after treatment with starch an amylase; and particularly after parenteral administration of certain proteins corresponding proteinases. These very significant findings met with numerous confirmations, but have been also strongly contested on the basis of conflicting experimental results.[122a-125] It must, however, be admitted that in the latter instances the experimental arrangements and methods employed differed frequently from those chosen by the original author. As to detailed explanations of these discrepancies, reference is made to the monographs of Abderhalden.[122, 126] The first practical application from a demonstration of protective ferments concerned the diagnosis of pregnancy, and was based on the assumption that during this condition chorionic elements, entering the maternal blood stream, would act as a protein foreign to the body and hence lead to the production of specific enzymes which split placenta protein. Actually, Abderhalden reported that in the serum of pregnant women enzymes could be detected, which caused a breakdown of placenta protein to dialysable and incoagulable split products. The same principle has been extensively elaborated for the examination of endocrine disturbances (thyroid, sex glands, etc.), and the basic idea was always the same—a pathology

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[120] W. Moppett: Med. J. Australia *1*: 684 (1934); J. Cancer Res. Comm., U. Sidney, *7*: 111 (1936).

[121] E. F. Thomson: J. Cancer Res. Comm., U. Sidney, *7*: 116 (1936).

[122] E. Abderhalden: Die Abwehrfermente, 1912; Die Abderhaldensche Reaktion, 1922; Lehrbuch der physiologischen Chemie, 1931; Abwehrfermente (Die Abderhaldensche Reaktion), Steinkopff, Dresden & Leipzig 1941.

122a. D. D. van Slyke: J. Biol. Chem. *23*: 377 (1915).
123. B. Oppler: Biochem. Ztschr. *75*: 211 (1916).
124. H. Kleinmann & G. Scharr: Biochem. Ztschr. *252*: 343 (1932).
125. P. Laurent-Gérard & H. Simmonet: Paris méd. *2*: 34 (1935).
126. E. Abderhalden: Handbuch der biologischen Arbeitsmethoden, Abt. IV, Lief. 415 (1933).

of these organs was assumed to entail a liberation of their proteins, which subsequently enter the humoral media and there elicit the formation of specific enzymes.

For the present purpose, it is not relevant to discuss the usefulness of these broader applications of Abderhalden's test, but those investigations are of greater interest in which attempts were made to demonstrate enzymes acting against tumor proteins in neoplasia, since they furnished a means of serological cancer diagnosis. The pertinent literature is enormous and may be grouped into three classes.

One, elaborating the theoretical viewpoints, a second, dealing with methodological problems, and the last, reporting on the practical results of the test. As to the theoretical concept of the reaction, Abderhalden himself appears to have modified his original views, according to which he saw in the defensive ferments, as the name implies, a rational reaction of the organism, facilitating the elimination of substances foreign to the body; more recently, however, the author also considered other possibilities, such as a formation of these enzymes actually from carried off and disintegrating cells. A point of preeminent importance for the whole problem is represented by the choice of a suitable method. The numerous changes which have been performed in this respect by the author himself, and also by many other workers, may be considered as the best indication that none of the proposed techniques have proved entirely satisfactory. The first procedure employed by Abderhalden was a method of dialysis; the cleavage products formed during the enzymatic digestion pass through a dialyzing membrane, and are shown to be present in the surrounding medium by means of a color test with ninhydrin. Of modifications devised later, the most important methods are the detection of the split products by means of refractometry,[127] or by determination of the incoagulable nitrogen according to the methods of Kjeldahl or Nessler, and by means of interferometry.[128] The last method may be regarded as the most sensitive one, but, nevertheless, yielded completely negative results in the hands of some investigators.[125] Every one of these methods acquired a different form in the work of the individual investigators. Thus the use of dialyzing membranes, occasionally found unreliable, has been abandoned and, instead, protein-free filtrates of the serum obtained by boiling and precipitation with colloidal iron hydroxide,[129] or by alcohol precipitation, have been used.[130] An interesting method has been developed in the "diasorcym test," in which tumor protein was artifically impregnated with iron salts, and the iron liberated as a consequence of protein cleavage was determined.[131] In connection with questions of technique, reference must be made to the great importance of employing an adequate tumor substrate for the test, and Abderhalden in particular stressed the necessity of testing each serum with several substrates, since he believed that the serum enzymes in question are specific for the type of neoplasm with which the particular patient is afflicted. Findings, confirming this view, have indeed been reported;[132, 133] that is, the serum, e.g., of a patient with gastric cancer was found capable of digesting protein derived from gastric cancer exclusively, or at least, the cleavage of this protein markedly exceeded that of other tumor proteins tested. This observation, if substantiated, would open a way for the diagnosis and localization of occult tumors, but at the same time must

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[127] F. Pregl & M. de Crinis: Fermentforsch. 2: 58 (1917).

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128. P. Hirsch: Ztschr. f. physiol. Chem. 31: 440 (1914); Fermentforsch. *1*: 33 (1914).

129. E. Freund, in K. H. Barrenscheen & R. Willheim: Die Laboratoriums-methoden der Wiener Kliniken, Deuticke, Vienna 1928, p. 383.

130. W. Lüttge & W. von Mertz: Die Alkoholextraktreaktion, Hirzel, Leipzig 1927.

131. Kottmann; quot. J. Grohde: Strahlentherapie *10*: 437 (1920).

132. H. Meyer: Biochem. Ztschr. *114*: 194 (1921).

133. K. Volkmann: Klin. Wchnschr. *5*: 837; 1563 (1926).

be regarded as a technical obstacle for practical cancer diagnosis, since it is hardly possible that one serum can be tested with protein substrates derived from all types of neoplasms.

By employing one or the other of the methods described, a great number of authors achieved more or less satisfactory results,[134-139] whereas others rejected the test in the light of their experiences[84, 140-144] and especially stressed the great technical difficulties and sources of error inherent in the methods.[145, 146] From the more recent literature, a study may be mentioned in which the interferometric method was used and fifty to seventy per cent of correct results were obtained in cancer patients, whereas thirty per cent of erroneous diagnoses appeared in tumor-free persons; this result implies neither a high sensitivity nor a specificity of the Abderhalden test, and, moreover, animal experiments with transplanted tumors and with tar cancer did not show the possibility of an early diagnosis.[147] Other authors reported analogous findings, namely, positive results in sixty per cent of the cancer patients tested, but succeeded in improving the sensitivity of the test by injecting a "reactin" into the patient prior to the performance of the serodiagnosis. It was assumed

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134. E. Frank & F. Heimann: Berlin. klin. Wchnschr. *50*: 631 (1913).
135. G. von Gambaroff: Munchen. med. Wchnschr. *60*: 1644 (1913).
136. R. S. L. Brockman: Lancet *2*: 1385 (1913).
137. Piorkowski: Berlin. klin. Wchnschr. *51*: 254 (1914).
138. S. Blackstein: Beitr. z. Geburtsh. u. Gynäk. *19*: 406 (1913–15).
139. M. Sanchez: Am. J. Cancer *19*: 40 (1933).
140. Oettinger, Fiessinger & Marie: Berlin. klin. Wchnschr. *51*: 1491 (1914).
141. E. Fränkel: Berlin. klin. Wchnschr. *51*: 356 (1914).
142. L. Michaelis & L. v. Lagermarck: Deutsche med. Wchnschr. *40*: 316 (1914).
143. B. Oppler: Deutsche med. Wchnschr. *43*: 1596 (1917).
144. E. Lendel: München. med. Wchnschr. *77*: 476 (1930).
145. F. Heimann & K. Fritsch: Arch. f. klin. Chir. *103*: 659 (1914).
146. S. Cytronberg: Mitt. a. d. Grenzgeb. d. inn. Med. u. Chir. *28*: 243 (1914).
147. A. Schmidt-Ott: Ztschr. f. Krebsforsch. *24*: 292 (1927).

that this injection enhanced the tumor decomposition, and accordingly led to an augmented liberation of the enzymes sought.[148] An improved effectiveness of the Abderhalden reaction was also lately claimed as resulting after a preliminary application of weak roentgen ray doses to the suspected tumor region.[149] A modification of the test has been devised by using a substrate, called "ultrapeptone" and consisting of polypeptides derived from hydrolyzed tumor protein,[150] instead of the native tumor protein, but favorable reports on the usefulness of this procedure[151, 152] are contrasted by entirely negative opinions.[153] Another author employed determinations of the pH in the mixtures of substrate and serum, before and after incubation, and believed that changes of the pH indicate the occurrence of protein cleavage,[154] but insufficient experiences to arrive at a definite conclusion have been collected with this technique.

A new approach for the detection of protective ferments has recently been devised by Abderhalden[155, 156] which consists, instead of using the serum, of the use of the urines of the cancer patients to be tested. The author reported he had obtained very satisfactory results in this manner, without however, presenting a detailed statistical analysis of the cases tested. Some authors[157, 158] announced successful application of this method to cancer diagnosis, and also found positive and specific reactions with the urines of rabbits inoculated with Kato sarcoma.[159] However, another author obtained only thirty-eight correct results in fifty cancer cases, whereas eight erroneous reactions occurred among 109 cancer-free persons tested, and the author therefore concluded that the test in the present form is not yet ripe for practical use.[160]

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[148] S. Beck & E. Lendel: Fermentforsch. *11*: 490 (1930).

[149] R. Merten & W. Spiegelhoff: Ztschr. f. klin. Med. *138*: 421 (1940).

[150] F. Figari: Arch. di biol. *7*: 3 (1930); in: A. Neumann: Streiflichter über das Carcinom, Vienna 1933, p. 37.

[151] B. D. Deliperi: Arch. di biol. *6*: 31 (1929).

[152] U. Rebaudi: Néoplasmes *9*: 99 (1930).

[153] A. Panagia: Policlinico (sez. med.) *40*: 213 (1933); abstr. Am J. Cancer *20*: 896 (1934).

[154] L. Thurner: Monatschr. f. Krebsbekämpf. *5*: 153 (1937).

[155] E. Abderhalden & H. Schenker: Fermentforsch. *16*: 14 (1938).

[156] E. Abderhalden: Forsch. u. Fortschr. *15*: 122 (1939).

[157] Y. Tanabe: Fukuoka Acta med. *32*: 28 (1939); abstr. Ztschr. f. Krebsforsch. *49*: Ref. 56 (1939).

[158] S. Tumo: J. Biochem. *32*: 371 (1940).

Carcinolytic reaction. This test goes back to the phenomenon of a lysis of cancer cells brought about by the action of normal serum under special experimental conditions, a discovery which was made by Freund and Kaminer.[161] These authors made the observation that isolated cancer cells, incubated with normal serum, are affected in such a way that microscopically a disappearance of a great percentage of the cells can be ascertained, whereas serum of cancer patients not only fails to induce this phenomenon, but is even endowed with the faculty of protecting cancer cells to a certain degree against the lytic action of normal serum. At the same time, and independently of Freund and Kaminer, an analogous phenomenon was described by Neuberg[162] who, working with large quantities of cancer tissue and normal serum, after digestion of this mixture, chemically determined an increase of the incoagulable nitrogen, which he assumed was due to a dissolution of cancer cells, resulting in the freeing of protein cleavage products. The further theoretical and diagnostic-clinical elaboration of this reaction, however, must be credited principally to Freund and Kaminer, and is described in the following.

These authors, from the very beginning of their studies on carcinolysis, started from the premise that a disposition to cancer is a determining factor in this malady, and made this idea the basis of their subsequent studies performed in the course of more than two decades.[163] Accordingly, they viewed the fact that normal serum dissolves cancer cells to be a protective mechanism of the body against cancer, and the fact that this ability is missing in the serum of cancer patients represents, in their opinion, the expression of a general disposition of these persons to neoplasia. Freund and Kaminer attempted by chemical methods to identify the factors responsible for the carcinolytic, or for the cancer-cell-protecting qualities of normal or cancer serum, respectively. In this connection, they reported first that extraction of normal serum with ether results in a loss of the carcinolytic power of the serum, whereas a strongly lytic substance was found to be present in the sodium bicarbonate extract of the ether extract. Further chemical work led the authors to the belief that the carcinolytic substance, thus isolated from normal serum, could be regarded as a saturated dicarboxylic acid of a molecular weight of 500. Fractionations of the serum indicated that this lytic substance is bound to the euglobulin fraction. In contrast with these findings in normal serum, an acid ether extract of cancer sera was found as containing an unsaturated dicarboxylic acid of a molecular weight of 550, which substance exhibits the same qualities as cancer serum, i.e., protects cancer cells against the action of lytic factors.[164] This unsaturated acid—termed by the authors as "cancer-cell-protecting substance"—is distinguished from the carcinolytic factor in that it is bound to the nucleoglobulin fraction of the serum. Hence the cancer serum differs from the normal one, not only by the absence of the lytic factor, but also by the presence of an anti-lytic factor. In spite of the fact that a definite chemical composition has been ascribed to these substances, several factors which have been established suggest that they are of an enzymatic nature, in particular the thermolability and the sensitivity to quinine, found inherent in the lytic factor.[163, 165, 166] At this point it seems appropriate to stress the fact that, according to Freund and Kaminer, the carcinolytic reaction makes it possible to differentiate between the two most important types of malignant tumors, i.e., between carcinoma and sarcoma. That is, serum of cancer patients exhibits the described qualities when reacting with carcinomatous cells, while it is capable of dissolving sarcomatous cells in the same way as normal sera, and vice versa, sera of sarcoma patients exert a lytic action on carcinomatous cells but not on sarcomatous ones. Due to the rarer incidence of sarcoma and especially on account of the difficulty of procuring a suitable cell suspension from sarcomas, most of the investigations enumerated in the following were limited to the carcinolytic phenomenon, as represented by the action of normal sera, or sera of carcinoma patients, on carcinomatous cells, and few experiences were collected regarding the action of serum of sarcoma patients.

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[159] S. Tumo: J. Biochem. *32*: 419 (1940).

[160] E. Tetzner: Ztschr. f. Krebsforsch. *50*: 465 (1940).

[161] E. Freund & G. Kaminer: Biochem. Ztschr. *26*: 312 (1910); Wien. klin. Wchnschr. *34*: 378 (1910).

[162] C. Neuberg: Biochem. Ztschr. *26*: 344 (1910).

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[163] E. Freund & G. Kaminer: Die biochemischen Grundlagen der Karzinomdisposition, Springer, Vienna 1925.

[164] E. Freund & G. Kaminer: Wien. klin. Wchnschr. *46*: 1576 (1933).

Freund and Kaminer furthermore developed a definite concept as to the point of origin of the substances responsible for the lytic or antilytic effect. They assumed their formation to take place in the intestinal tract. In cancer-free persons, and especially in those who do not possess a disposition to cancer, intestinal bacteria were believed to produce the normal saturated fatty acid from ingested fat (palmitin, stearin) whereas in the intestinal tract of persons disposed to neoplasia, or in that of cancer patients, the same nutritional material should undergo—by bacterial action—a conversion

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165. R. Willheim & K. Stern: Wien. klin. Wchnschr. *43*: 227 (1930).

166. O. Deutschberger, R. Königstein, E. Paschkes, K. Stern & R. Willheim: Enzymologia *5*: 347 (1938).

into the pathological unsaturated dicarboxylic acid, i.e., the "cancer-cell-protecting-substance." The carcinolytic factor has been demonstrated as present not only in blood serum, but also in extracts of most normal organs, especially abundant in the thymus,[167] a fact which has been mentioned in chapter VIII. Particular significance has been assigned by the authors to the findings that the carcinolytic property was lacking in organs or tissues which are considered as changed in a precancerous manner, e.g., in gastric ulcer, in ulcus cruris and in the skin of mice painted with tobacco extracts.[168] These observations served as a basis for the assumption of a local disposition to neoplasia. In accordance with the theory that the substances in question are formed in the intestinal tract by the action of bacteria, it seemed plausible to look for differences in the intestinal flora, and such endeavors apparently met with success, inasmuch as the authors reported that strains of Coli bacteria isolated from the intestinal contents of normal persons can be differentiated from those cultivated from feces of cancer patients.[169, 170] This finding has likewise been utilized for diagnostic purposes, and was corroborated insofar as other workers detected differences between colonies of Coli strains, obtained from feces of tumor-bearing mice or of cancer patients, and those derived from normal animals or humans, as far as their ability to ferment certain carbohydrates (sionon) was concerned.[171] Collaborators of Freund, furthermore, were able to demonstrate similar changes in the intestinal flora of tumor-bearing animals (rabbits with Brown-Pearce carcinoma, mice with spontaneous tumors, or with Ehrlich carcinoma). As compared with the normal intestinal flora, the bacteria cultivated from feces of tumor-bearing animals showed differences similar to those exhibited by the microorganisms in the intestinal tracts of normal humans, and of cancer patients.[172] Later studies, however, arrived at divergent results. Since abnormal Coli strains were found in about forty-five per cent of the cancer patients tested, and in twenty-six per cent of cancer-free persons, it was concluded that these changes cannot be regarded as being specific for neoplasia.[173] Similarly it has been suggested by other authors, who observed only irregular changes of the intestinal flora in cancer patients, that these phenomena cannot be considered as being primarily connected with the disease, but are rather of secondary nature, due to metabolic alterations.[174] Hence the causal link, inferred by Freund and Kaminer as existing between intestinal flora, ingested fat, carcinolytic or antilytic factors, on the one hand, and etiology of, or disposition to cancer, on the other, cannot be accepted as definitely proven, a fact which, however, does not detract from the validity of the carcinolytic phenomenon itself. As to therapeutic, principally dietary, implications, which have been derived by Freund from his theory, reference is made to the presentation of this problem in chapter V.

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[167] G. Kaminer & O. Morgenstern: Biochem. Ztschr. *84*: 281 (1917).

[168] E. Freund & G. Kaminer: Biochem. Ztschr. *112*: 124 (1920).

[169] E. Freund & G. Kaminer: Wien. klin. Wchnschr. *43*: 993 (1930).

[170] A. E. Klein: Wien. klin. Wchnschr. *46*: 1587 (1933).

[171] F. Silberstein, F. Rappaport & E. Kolmer: Zentralbl. f. Bakt. (Abt. 1) *124*: 32 (1932).

Returning to the discussion of the carcinolytic test proper, its technical performance may be briefly outlined here, whereas precise data may be gained from the original articles, or from text books.[175] A suspension of cancer cells is prepared from human tumors (usually liver metastases) and, to one volume of this substrate, twenty volumes of the serum to be examined are added. By means of a counting chamber, the cell concentration of this mixture is determined immediately; the specimen is incubated at 38° C for 12 to 24 hours, and following this period a second microscopical count is performed. If serum of a cancer-free person has been used, the second cell count reveals a reduction of the cell concentration which, expressed in percentages of the first count, may range between twenty-five and sixty per cent, whereas in the case of a cancer serum, the lysis determined microscopically, as described above, is either completely missing or is below twenty per cent. In addition to this different behavior, sera of cancer patients exhibit another distinguishing property, apparent in the so-called "protective test"; that is, a mixture of a normal serum with a cancer serum in the proportion of 2:1, used for a carcinolytic test in the manner described, demonstrates the absence of a lytic effect. Instead, or in addition to, the testing of the serum, it was also proposed to cultivate Coli strains from the feces of the patients and, in accordance with the conceptions described above, it was reported that carcinolytic factors could be detected in the media of "normal" Coli cultures, whereas the cancer-cell-protecting factor was demonstrable in colonies of bacteria derived from cancer patients. Favorable experiences with this test, based on an investigation of 500 cases, have been published.[176]

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[172] B. Lustig & E. Kolmer: Ztschr. f. Krebsforsch. *46*: 44 (1937).

[173] R. Goiffon & H. Prétet: Arch. d. mal. de l'app. digestif *27*: 869 (1937).

[174] C. Manzini & G. F. Giardini: Bol. ist. sieroterap. milan. *16*: 150 (1937); abstr. Ztschr. f. Krebsforsch. *47*: Ref. 324 (1938).

[175] E. Freund; in: K. H. Barrenscheen & R. Willheim: Die Laboratoriums methoden der Wiener Kliniken, Deuticke, Vienna 1928, p. 370.

The clinical results, obtained by the authors of the tests, have been very satisfactory, inasmuch as a margin of error of approximately only fifteen per cent was observed, both in the sera of cancer-free persons and of cancer patients. In the latter respect, it was found that far advanced cancer cases frequently yielded negative reactions, that fever in cancer

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176. M. Plonskier & R. Cyterman-Kon: Wien. klin. Wchnschr. *50*: 1526 (1937).

patients caused the appearance of a lytic quality in the serum, and that sera of skin cancer patients almost regularly exhibited carcinolytic property. On the other hand, the apparently erroneous results obtained in cancer-free persons were thought to be due to a disposition to neoplasia, inherent in those persons at a time when clinical tumors could not as yet be found. It is obvious that by thus stamping the carcinolytic test as a means of detecting a disposition to cancer, its clinical value is, at least at present, rather reduced. Numerous reinvestigations of the carcinolytic reaction have been carried out, and while some studies arrived at results comparable to those of the original authors (correct findings in seventy to eighty-five per cent of the cases),[177-182] other workers gathered divergent results, causing them to deny a practical value of the test [183] or even to doubt the theoretical foundation of the phenomena.[184, 185] Possible sources of error in cancer-free persons have been pointed out, such as the presence of lues,[186] or reduced carcinolytic power of sera of patients in the older age group.[187, 188] Sera of patients with skin cancer were rarely found to yield positive reactions, frequently they even exhibited an increased carcinolytic activity.[189] The writers themselves had occasion to devote a great deal of work to the study of the carcinolytic reaction and, on the basis of ex-

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177. A. Stammler: Wien. klin. Wchnschr. *24*: 774 (1911).
178. P. v. Monakow: München. med. Wchnschr. *58*: 2207 (1911).
179. L. Arzt & W. Kerl: Wien. klin. Wchnschr. *25*: 1821 (1912).
180. R. Kraus, E. v. Graff & E. Ranzi: Wien. klin. Wchnschr. *24*: 1003 (1911).
181. N. Waterman & L. de Kromme: Biochem. Ztschr. *182*: 377 (1927).
182. E. Jents: Monatschr. f. Hals-, Nasen- u. Ohrenh. *71*: 92 (1937).
183. K. Frankenthal: Ztschr. f. Krebsforsch. *17*: 253 (1920).
184. C. E. Simon & W. S. Thomas: J. A. M. A. *51*: 915 (1908).
185. G. M. Fasiani: Biochim. e terap. sper. *8*: 205 (1912).
186. G. Montemartini: Tumori n.s. *2*: 1 (1928).
187. K. Nather: Klin. Wchnschr. *1*: 2166 (1922).
188. G. Pacetto: Tumori *11*: 421 (1925).
189. F. Flarer: Riforma med. *42*: 1083 (1926).

periences collected during more than ten years, there is no doubt in their minds regarding the theoretical validity of the phenomenon in question, i.e., that a basically different action of normal serum, or of serum of cancer patients, on cancer cells in vitro is evident.[cf. 190] If, however, other authors drew different conclusions from their investigations, in our opinion, this may be explained as being due to two complicating factors, which are involved in the practical performance of the reaction. One of these obstacles lies in the morphological character of the method, i.e., the microscopical cell count which necessarily entails sources of subjective errors, particularly if the investigators have not acquired sufficient practical experiences. Another aggravating factor of still greater importance is represented by the difficulty of obtaining a cancer-cell material suitable for the performance of the test, and especially by the fact that the adequacy of the substrate is only of limited duration, necessitating a frequent control of the cells with sera of known cancer patients, and of cancer-free persons. In fact, other authors have also interpreted their experiences with the carcinolytic test in a similar light. In one of these studies, performed on a very broad scale, 1007 normal sera, 473 sera of pregnant women and 219 sera of cancer patients were examined. Although in the latter only fifteen per cent of correct results were found, and hence a practicability of the test had to be denied, the authors stated; "Doubtless it would be more nearly correct to say that in but fifteen per cent of the cases it was possible to prepare for the test a suitable cell suspension" [191] and "accumulating data but added to the conviction that such sera (i.e., of cancer patients) possessed properties which, in their action on cancer cells, distinguished them from normal

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190. K. Stern & R. Willheim: Ztschr. f. Krebsforsch. *46*: 379 (1937).

191. G. H. Smith, E. F. Jordan & F. B. Mack: Yale J. Biol. & Med. *8*: 603 (1936).

sera."[192] Similarly, another worker inferred from his results that they do not warrant a clinical application of the test, but he maintained the existence of an essential difference between normal and cancer sera as expressed in the carcinolytic reaction.[193]

In view of these circumstances, attempts have frequently been made to adapt the test to experimental conditions more favorable to a practical utilization. In this connection, efforts were made to divest the reaction of its morphological nature, and to endow it with an objective character, by substituting chemical or physicochemical procedures for the microscopical cell count. As mentioned before, an increase of the incoagulable nitrogen has been found as a consequence of carcinolysis occurring,[168] but a clinical application of this method appears impracticable because of the large quantities of serum required. It has been proposed to use refractometry for the determination of carcinolysis, and good results were reported from this procedure,[194, 195] but other authors failed to concur.[187, 196] Investigations performed by the writers of this book suggested that the point of attack of the carcinolytic factors is to be seen in the lipid structure of the cancer cells. Based on this fact, which was substantiated by the chemical finding of a lipid increase taking place in serum parallel to its carcinolytic action, a colorimetric estimation of carcinolysis has been devised by staining serum, after its action on cancer cells, with an aqueous solution of neutral red.[197] Furthermore, a clearer insight into the mechanism of carcinolysis has been gained, inasmuch as it was disclosed that the absence of lytic power is apparent in serum of cancer patients, only when the original technique of Freund and Kaminer is followed, i.e., when twenty volumes of serum are added to one volume of cell suspension. When, however, other volumetric proportions between cancer cells and serum (1 :1, 1 :5, 1 :10) are chosen, a lytic quality may also be detected in cancer serum. In contrast to normal serum, where increase of the serum volume is followed by an augmented carcinolysis, the lytic activity of cancer serum decreases with increasing serum quantities employed.[198] This peculiar behavior was interpreted as indicating that lytic factors are present also in the cancer serum, but are paralyzed by an antilytic factor, which latter substance is characterized by a high adsorbability to cancer cells and, if present in large enough concentrations, protects them against the action of lytic factors.[190, 198] Utilizing these findings, a modification of the carcinolytic test has been developed. By testing the serum with cancer cells in four different volumetric proportions (cells: serum –1 :1, 1 :5, 1 :10, 1 :20), the carcinolytic effect was measured in these four samples, both by cell counts and by the objective colorimetric method mentioned. The results were plotted in the form of a curve which as a rule exhibits, in the case of normal sera, a rising tendency; in the case of cancer sera, a falling one. By using these procedures, and provided that suitable cell suspensions are available, according to our experience optimal results of approximately eighty per cent of correct results are obtainable.[199] One re-examination of this method, which led to negative results,[200] has been found to be inconclusive on account of essential deviations from the original technique.[201] Another author obtained almost one hundred per cent correct results in cancer cases with the same

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[192] G. H. Smith & F. B. Mack: Yale J. Biol. & Med. *9*: 172 (1936–37).

[193] G. A. Kreuzwendedich von dem Borne: Nederl. tijdschr. v. geneesk. *81*: 4664 (1937).

[194] R. Koritschoner & O. Morgenstern: Biochem. Ztschr. *104*: 259 (1920).

[195] R. Koritschoner: Biochem. Ztschr. *129*: 605 (1922).

[196] K. Nather & V. Orator: Mitt. a. d. Grenzgeb. d. inn. Med. u. Chir. *35*: 611 (1922).

[197] R. Willheim & K. Stern: Biochem. Ztschr. *226*: 315 (1930).

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198. R. Willheim & K. Stern: Wien. klin. Wchnschr. *43*: 227 (1930).
199. R. Willheim & K. Stern: Wien. klin. Wchnschr. *44*: 728 (1931).
200. V. Carminati: Biochim. e terap. sper. *18*: 181 (1931).
201. R. Willheim & K. Stern: Biochim. e terap. sper. *20*: 253 (1933).

method, but also erroneous diagnoses in about forty per cent of cancer-free persons,[202] findings which—in our opinion—may have been due to a deficiency of the cell suspension used. In a more recent study ninety per cent correct findings were obtained in thirty cancer cases by using the colorimetric estimation of carcinolysis,[203] but no experiences with this test in cancer-free cases have been reported by this author.

Endeavors aimed at removing another obstacle in the performance of the carcinolytic test were concerned with securing a more reliable and stable substrate than the cancer cell suspensions hitherto used. In this respect, several authors believed that cell suspensions prepared from animal tumors were preferable to those derived from human neoplasms,[181, 204, 205] but our own experiences, and also the findings of other workers,[206] could not confirm the existence of such an advantage. As mentioned above, it has been recognized that the lipid cell structure is principally involved in the carcinolytic effect, and that a liberation of lipids from the cancer cells accompanies their lysis. Consequently, attempts were made to replace the cancer cell suspensions with lipid extracts derived from tumor tissue.[207] These experiments led to interesting results, but were not pursued to a point making a practical application possible. Another investigator, however, proceeded from these findings, and elaborated a method which closely resembles the modification described above,[199] and he determined the action of serum on alcoholic tumor extracts by means of interferometry, a procedure which yielded very satisfactory results to the author.[208] An interesting modification of the carcinolytic test has been based on the observation that, instead of suspensions of cancer cells, liver cells prepared from metastases-free organs of cancer patients may be successfully employed.[209] This finding has been confirmed,[210] but another worker found this substrate unsuitable, at least when he used it for the colorimetric estimation of carcinolysis.[208] Further examinations of this question are desirable, not only because—if substantiated—liver cells of cancer patients would represent a much more easily accessible substrate, less affected by regressive changes, but also because of a theoretical reason. This would prove that changes of the cell structure, making cancer cells subject to a lytic action of normal serum, are not confined to the tumors proper, but extend also to other organs, a concept which is in accord with the trend of considering neoplasia not as a merely local disease but as one affecting the whole body. In this connection, it must be stressed that cells of normal adult or embryonic organs are not influenced in a different manner by sera of cancer-free persons or of cancer patients. In particular, it has recently been reported that no difference was observed with regard to the cytolytic activity of normal or of cancer serum, acting on chick embryo cells, but the degree of cytolysis increased with advancing differentiation of the cells.[211] A utilization of a modified carcinolytic test (Klein test) was undertaken on a very broad scale several years ago; the cell suspensions were prepared from animal tumors, microphotograms were intended to facilitate and objectivate the necessary cell count, and it was stated that for improving the effectiveness of the test it is necessary to exclude sera of patients exposed to a great variety of "interfering factors" (roentgen radiation, fluoroscopy, surgery, administration of certain drugs) since these procedures were claimed to change the serum behavior in an unspecific manner.[212] By means of such methods, tens of thousands of sera have been tested in a central institute but, while the first reports recorded highly satisfactory results,[218-220] soon afterwards experiences were collected which evoked the greatest doubt as to the reliability and efficiency of the method, since especially "blind test series" yielded erroneous diagnoses in a very high percentage,[221-230] and consequently this method of cancer diagnosis has been completely abandoned. A phenomenon which may be regarded as related to carcinolysis has been observed by using tumor cells fixed in alcohol. When this substrate was exposed to the action of normal serum for twenty-four hours, and the cells were subsequently stained with basic and then with acid stains, it was found that the

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[202] F. P. Tinozzi: Diag. e tec. di lab. *3*: 541 (1932).

[203] G. Perazzo: Tumori n.s. *13*: 177 (1939).

[204] R. Kraus & E. v. Graff: Wien. klin. Wchnschr. *24*: 191 (1911).

[205] J. Flaszen & H. Wachtel: Klin. Wchnschr. *15*: 684 (1936).

[206] H. Auler: Neue deutsche Klin. *5*: Erg.-B., p. 187 (1937).

[207] R. Willheim & K. Stern: Biochem. Ztschr. *239*: 473 (1931).

[208] M. W. Mettenleiter: Bol. liga contra el cancer *13*: 165 (1938); Nature, London *145*: 305 (1940).

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[209] K. Bauer: Wien. klin. Wchnschr. *48*: 1409 (1935); *49*: 266 (1936).
[210] R. Königstein & R. Willheim: Biochem. Ztschr. *292*: 276 (1937).
[211] W. Maasen: Ztschr. f. Immunitätsforsch. u. exper. Ther. *95*: 514 (1939).

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212. G. Klein: Wissenschaftl. Woche, Frankfurt a. M., Vol. *2*: 40 (1934); Arch. f. klin. Chir. *183*: 194 (1935).
213. L. Simon: Wissenschaftl. Woche, Frankfurt a. M., Vol. *2*: 50 (1934).
214. L. Simon, H. Maxon & F. Wittenbeck: Ztschr. f. Krebsforsch. *42*: 347 (1935).
215. J. Kretz & A. F. Pellegrini: Wien. klin. Wchnschr. *48*: 1446 (1935).
216. Hummel: Deutsche Ztschr. f. Chir. *245*: 332 (1935).
217. C. Reimers: München. med. Wchnschr. *83*: 1375 (1936).
218. F. Grögler: München. med. Wchnschr. *83*: 1377 (1936).
219. G. Hepp: München. med. Wchnschr. *83*: 1378 (1936).
220. R. Klapp & J. Baumann: Zentralbl. f. Chir. *63*: 2162 (1936).
221. C. Barthels: Arch. f. klin. Chir. *183*: 203 (1935); Beitr. z. klin. Chir. *163*: 624 (1936).
222. H. Horster: Deutsche med. Wchnschr. *62*: 460 (1936).
223. B. Karitzky: Beitr. z. klin. Chir. *163*: 618 (1936).
224. Kirschner: Arch. f. klin. Chir. *183*: 23 (1935).
225. O. Stör & K. Pfeil: Beitr. z. klin. Chir. *163*: 630 (1936).
226. L. Simon & F. Wittenbeck: München. med. Wchnschr. *83*: 2000 (1936); Ztschr. f. Krebsforsch. *45*: 265 (1937).
227. Lönne: München. med. Wchnschr. *83*: 2077 (1936).
228. H. Nasemann & E. Weber: München. med. Wchnschr. *84*: 255 (1937).
229. A. Fehr: Schweiz. med. Wchnschr. *67*: 8 (1937).
230. A. Liepelt: Monatschr. f. Krebsbekämpf. *5*: 96 (1937).

basic dyes did not take, whereas treatment of the cells with serum of cancer patients resulted in both acid and basic staining. This different behavior of normal and of cancer serum was assumed to indicate that the former contained factors which destroyed the acid albumin in the cancer cells, while such factors were absent in sera of cancer patients.[231] To what extent these reactions may be utilized for practical purposes of cancer diagnosis cannot as yet be decided, because of insufficient experiences collected with this procedure.

In addition to its diagnostic application, the phenomenon of carcinolysis gave rise to a large number of studies, less concerned with practical purposes than with general biological aspects. In this connection several supplementary findings may be mentioned with regard to the occurrence of the lytic and the antilytic factors. Investigations of animal sera showed the carcinolytic substance to be present in sera of rabbits and guinea pigs, but not of rats and goats.[204] Confirming the findings of Freund and Kaminer, other workers also demonstrated carcinolytic effects in organ extracts,[232] especially in extracts of lymph nodes and of spleen.[233, 234] In agreement with the finding mentioned above, regarding the presence of carcinolytic factors also in the serum of cancer patients,[198] other authors arrived at similar results by means of different experimental arrangements.[235–237] It appears especially noteworthy that carcinolytic substances have been detected in autolysates of tumors, and that even a specific character was assigned to these lysins, inasmuch as their cytolytic

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231. A. Hirschfeld: Arch. inst. prophylactique *8*: 307 (1936).

232. N. Waterman: Biochem. Ztschr. *188*: 65 (1927).

233. G. Fichera: Tumori, Hoepli, Milano 1933.

234. M. Ferro: Tumori n.s. *6*: 1 (1932).

235. N. Waterman, L. de Kromme & J. F. Lemmens: Biochem. Ztschr. *205*: 1 (1929).

236. A. Klopstock & H. Lehmann-Facius: Klin. Wchnschr. *7*: 1085 (1928).

237. S, Komiya: Nagoya J. Med. Sc. *4*; 59 (1930).

effect proved strongest when acting on cancer cells of the same type as the tumor used for preparing the autolysate. This observation suggested the assumption of a relationship of these lysins with bacteriophages.[238] Even outside of the animal organism, carcinolytic extracts have been obtained from lemons, prunes and potatoes.[239]

Abundant quantities of the antilytic factors ("cancer-cell-protecting substance" of Freund and Kaminer) have been demonstrated in ascites fluid of cancer patients[165] and also in ascites of mice with Ehrlich carcinoma.[240] The cerebrospinal fluid of cancer patients was found to contain antilytic factors, whereas spinal fluids of cancer-free persons did not exhibit a carcinolytic property.[241] A biological significance of antilytic factors has been made very probable on the basis of several reports. Thus these substances were found to stimulate the growth of epithelial elements in tissue cultures, while inhibiting that of connective tissue,[240] and treatment of tumor cells previous to their inoculation with serum of cancer patients augmented their virulence.[242, 243] It has been mentioned in detail in chapter VIII, that a number of findings established a close relationship of the carcinolytic phenomenon with the function of the reticulo-endothelial system,[232, 235, 244, 245] and this assumption has been put on a more solid basis by the experimental observation that sera of normal animals acquire the characteristic behavior of cancer sera, when damages of the reticulo-endothelial system were produced in the animals.[246] Hence it was concluded that a normal

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238. A. Edelmann, L. Schönbauer, & W. Schloss: Wien. klin. Wchnschr. *42*: 1245 (1929).
239. V. Laufberger: Časop. lék. česk. *66*: 172 (1927).
240. N. Waterman: Wien. med. Wchnschr. *85*: 405 (1935).
241. B. Lustig: Wien. klin. Wchnschr. *46*: 1581 (1933).
242. H. Hirschfeld: Ztschr. f. Krebsforsch. *11*: 388 (1912).
243. K. Stern & R. Willheim: Ztschr. f. Krebsforsch. *39*: 54 (1933).
244. G. C. Peracchia: Ztschr. f. Krebsforsch. *26*: 42 (1928).
245. H. Zacherl: Klin. Wchnschr. *9*: 289 (1930).
246. R. Willheim & K. Stern: Ztschr. f. Krebsforsch. *38*: 501 (1933).

reticulo-endothelial function is necessary for maintaining the normal carcinolytic qualities of the serum, and that the appearance of antilytic factors in the body is the expression of an impaired reticulo-endothelial activity, a view, according to which the carcinolytic test would have to be regarded as a functional test of the reticulo-endothelial system. A dependence of carcinolytic phenomena on endocrine factors has been suggested by the finding that the addition of anterior pituitary extracts to normal serum inhibited its lytic activity, whereas posterior pituitary extracts counteracted the effect of cancer serum, i.e., of protecting cancer cells against lytic factors. By themselves, these endocrine preparations did not affect cancer cells in vitro, but simultaneous addition of both hormones to serum cancelled each other.[247]

Regarding the chemistry of the carcinolytic and antilytic factors, the statements of Freund and Kaminer, mentioned above, could not be confirmed in later investigations, that is, their configuration of a saturated, or an unsaturated, dicarboxylic acid has been denied.[248-249] Although the carcinolytic factors could not be identified with any of the known enzymes,[285] a definite relationship of the lytic effect to enzymatic processes has been pointed out, in particular a connection was assumed to exist between carcinolysis and lipase-activators, isolated from mesenchymal tissues.[250] Another author was inclined to view carcinolysis merely as an autolytic phenomenon, not requiring an active participation of the serum,[251] but the exact comparison of the kinetics of autolytic and carcinolytic effects demonstrated an essential difference of these two processes.[21d] Likewise, a concept which regarded the carcinolytic effect as mainly due to physicochemical factors, i.e., to changes of the electrolyte concentration and of

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247. B. Lustig & H. Wachtel: Klin. Wchnschr. *17*: 163 (1938).
248. N. Waterman & L. de Kromme: Biochem. Ztschr. *188*: 65 (1927).
249. A. v. Wacek & O. Pesta: Ztschr. f. Krebsforsch. *44*: 345 (1936).
250. N. Waterman: Ztschr. f. Krebsforsch. *34*: 327 (1931).
251. W. Raab: Klin. Wchnschr. *14*: 1633 (1935).

the osmotic pressure,[252] does not seem acceptable, on account of deviations from the original technique and because of theoretical considerations.[190] Attempts have been made to explain the carcinolytic phenomenon as being of an immunological nature,[236] and similarly some authors stressed that, according to their experiences, carcinolysis required the presence of active complement.[191, 206] Whereas the problem of the nature of the carcinolytic factors is undoubtedly not clarified, it may be repeated that our own investigations devoted to this question suggested the attributing of enzymatic qualities to the carcinolytic factors, which appear principally to attack the lipid structure of the cancer cells as evidenced by a liberation of lipid substances from cells subjected to the lytic action of serum.[190]

A great deal of work has more recently dealt with the chemical definition of the antilytic factors present in the serum of cancer patients and since, of various synthetic products tested, cholesterol-n-butyrate was found to protect cancer cells against lytic effects, this substance was held to be identical with the natural factor.[253] The potency of this cholesterol ester was shown to be strongly activated by ergosterol or vitamin D,[254] and evidence was presented, which should prove that serum of cancer patients, but not of cancer-free persons, is capable of the enzymatic synthesis of cholesterol-n-butyrate from added cholesterol and butyric acid.[255] On the other hand normal serum was reported to contain a "de-activator" of cholesterol-n-butyrate,[254, 256, 257] and this factor was considered as an oxidative enzyme (dehydrogenase), which is present in the blood or tissue of normal persons but not of cancer patients.[258, 259] Hence, this theory established a connection between the characteristic behavior of serum, as expressed in the carcinolytic reaction, and between disturbances of the oxidative metabolism, which have been shown to exist in neoplasia (cf. chapter VI). In spite of the extensive work leading to interesting theoretical conclusions, objections have been raised against the fundamental premise of these conceptions, that is, of an identity of the natural antilytic factor with cholesterol-n-butyrate. Although reinvestigations proved that this latter substance, when added to normal serum in minute quantities, protects cancer cells against lysis,[190] certain chemical and biological differences have been pointed out as existing between the antilytic factor of cancer serum, and the cholesterol ester,[260] and it was especially stressed that a great number of organic compounds could be demonstrated to act as antilytic factors in a similar manner as cholesterol-n-butyrate.[190, 261, 262] Therefore, the findings obtained with this substance must at present be considered merely as very interesting model experiments, whereas definite proof for the identity of the antilytic factor with cholesterol-n-butyrate could be furnished only by chemical isolation of this substance from sera of cancer patients, an undertaking of a difficulty which is obviously not to be underrated. Interesting results have been obtained by means of physicochemical methods applied to the problem of carcinolysis; electrophoresis of normal or of cancer serum resulted in an accumulation of lytic factors at the anode,

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[252] A. v. Wacek & O. Pesta: Ztschr. f. Krebsforsch. *46*: 211 (1937).

[253] A. v. Christiani: Ztschr. f. Krebsforsch. *41*: 25 (1935); *42*: 25; 317 (1935); *44*: 467 (1936).

[254] A. v. Christiani: Ztschr. f. Krebsforsch. *46*: 292 (1937).

[255] A. v. Christiani & H. Morth: Ztschr. f. Krebsforsch. *46*: 300 (1937).

[256] A. v. Christiani & L. Hofmann: Ztschr. f. Krebsforsch. *47*: 188 (1938).

[257] A. v. Christiani: Ztschr. f. Krebsforsch. *49*: 221 (1939).

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258. E. Maier & A. v. Christiani: Ztschr. f. Krebsforsch. *49*: 679 (1940).
259. A. v. Christiani: Ztschr. f. Krebsforsch. *48*: 369 (1939).
260. B. Lustig: Ztschr. f. Krebsforsch. *43*: 246 (1936); *45*: 480 (1937).
261. B. Lustig & H. Wachtel: Biochem. Ztschr. *271*: 345; 357 (1934); Ztschr. f. Krebsforsch. *41*: 468 (1935).
262. B. Lustig & H. Wachtel: Ztschr. f. Krebsforsch. *43*: 343 (1936).

whereas the antilytic factors of sera of cancer patients migrated to the cathode, and furthermore a separation of these two factors was also accomplished by means of specific adsorption and elutions.[166] Continued work in this direction may decide whether these procedures may be utilized for purifications and chemical identification of these substances.

The problem of carcinolysis has undergone only a comparatively meager elaboration as far as experimentation with tumor-bearing animals is concerned. A decrease of the carcinolytic activity was observed in sera of animals treated with tar [263] and mice inoculated with tumors. In the first period an increase, but later, parallel to advancing tumor growth, a decrease of the carcinolysis took place.[200] Antilytic factors have been isolated from colonies of intestinal bacteria of tumor-bearing rabbits or mice, and the properties of these substances were generally found to correspond to those derived from intestinal contents of cancer patients, with the exception that the substances obtained from animals failed to elicit cutaneous reactions in tumor-bearing animals or humans.[264] The behavior of more than 100 various organic compounds in the carcinolytic test has been used as a criterion for their possible chemotherapeutical value and, accordingly, the administration of these substances was tested as to their influence on animal tumors; [261, 262, 265] most of these findings have been mentioned in chapter II.

Several investigations dealt with the influence of physical energies on the carcinolytic qualities of serum. It has been claimed that radium or roentgen irradiation exert, in this respect, the opposite effects, inasmuch as the latter caused, in vitro, formation of antilytic factors, whereas the former

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263. F. Piccaluga: Tumori *11*: 45; 265; 291 (1925).

264. B. Lustig, K. Pichler & M. Bodian: Ztschr. f. Krebsforsch. *46*: 24 (1937).

265. B. Lustig & H. Wachtel: Bull. Assoc. franç. p. l'étude du cancer *25*: 542 (1936).

produced lytic factors.[164, 266] Another author found that roentgen irradiation of lytic factors resulted in an intermittent effect, i.e., varying doses and times of radiation increased or decreased the lytic activity in a sine-wave-like manner.[232] More recently it has been established that exposure of the serum of cancer patients to a roentgen dose of 15,000r results in a destruction of the antilytic factors, as indicated by carcinolytic tests performed prior to and after the irradiation.[166] Likewise, it was found that β-radium irradiation of maleic acid, a substance which strongly protects cancer cells against lysis, destroys this property, and the irradiated product even cancels the antilytic effect of non-irradiated maleic acid.[267] A conversion of antilytic factors into lytic substance has been claimed to be obtainable also by vitro exposure of the serum of cancer patients to ultra short wave radiation.[268] In vivo, roentgen irradiation of the spleens of humans was found to cause an increase of the carcinolytic serum activity when small (stimulating) doses were applied, whereas large (paralyzing) roentgen doses led to a weakening of the carcinolysis.[245] These findings may again call attention to the relationship of carcinolysis with the reticulo-endothelial function, a problem discussed in chapter VIII, and a similar interpretaton may be valid for the observation that high-frequency irradiation in vivo was followed by an increase of the carcinolytic power of the sera of the treated patients.[269]

Proteolytic enzymes. With this group of tests, we approach diagnostic methods which, in contrast to the Abderhalden test, or to the carcinolytic test, are based on enzymatic processes of a more clearly defined nature.

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[266] E. Freund: Wien. klin. Wchnschr. *46*: 1576 (1933).

[267] B. Lustig & H. Wachtel: Biochem. Ztschr. *298*: 330 (1938).

[268] G. Fuchs: Klin. Wchnschr. *15*: 759 (1936); Strahlentherapie *57*: 636 (1936).

[269] S. Knopfmacher: Wien. klin. Wchnschr. *46*: 220 (1933).

Fibrinolytic test of Fuchs. In the course of investigations on the protease content of sera of animals and humans, Fuchs[270] made observations which revealed a phenomenon of a hitherto unknown nature. This may be expressed principally in the rule that the individual sera were found capable of splitting fibrin, which is foreign to the species, but not fibrin of the same species. Moreover, a possibility for a practical utilization of this finding appeared to be given by the fact that the serum of persons affected with certain pathologies—particularly with neoplasia, tuberculosis or lues—seemed to have acquired a new property, i.e., these sera also brought about a cleavage of species-specific fibrin, provided that the fibrin was not derived from a person afflicted with the same disease as the donor of the serum. Conversely, fibrin prepared from the blood of a patient suffering from one of the maladies named, was split by human serum, provided that this was derived from a person free of the disease of which the donor of the fibrin employed was suffering. To illustrate this behavior, it may be explained that sera of cancer patients cause a cleavage of fibrin derived from cancer-free humans, and also of fibrin from patients afflicted with lues or tuberculosis, but not of fibrin prepared from blood of cancer patients. On the other hand, this latter substrate is split by sera of all individuals of the same species, exclusive of those diseased with neoplasia. The fibrinolysis occurring in these experiments has been measured by determinations of the non-protein-nitrogen of the serum, before and after interaction with the fibrin substrate. A colorimetric procedure, especially adapted to this purpose and capable of indicating small reaction values, has been used[271] and in later work the author replaced the fibrin substrate with a protein obtained from a serum by means of precipitation with trichloracetic acid.[272] As to the theoretical background of fibrinolysis, the author assumed a connection to exist with immunological phenomena, since he observed that interaction of "cancer fibrin" and of cancer serum (or generally of substrate and of serum derived from patients afflicted with the same pathology) not only failed to yield an increase of incoagulable nitrogen, but even resulted in a decrease of this nitrogen fraction. This would indicate that the substrate in use had adsorbed protein derivatives of lower molecular weight from the serum, and a similar phenomenon was apparent when antigens and antibodies of known nature reacted with each other.[273] The experiences which the author of the method collected on the basis of more than three thousand five hundred cases have been extremely favorable; almost one hundred per cent correct diagnoses were reported.[274] The results of continued work on the theoretical side of the fibrinolysis and material on its diagnostic value were published in later papers of the same author.[275] Some of the older studies employing this test for diagnostic purposes stressed the fact that, although correct results were obtained in a very high percentage of cancer sera, erroneous diagnoses appeared in thirty or more per cent of sera of tumor-free patients,[276-278] and some workers completely failed to note any characteristic behavior of normal or cancer sera in the fibrinolytic test.[279-280] Fuchs, however, answered these objections by pointing out that the results mentioned had been due to the use of unsuitable protein or fibrin substrates.[281] While thus the rather unfavorable experiences of earlier work may have been due to technical difficulties, a great many studies of more recent date have confirmed the theoretical validity of the fibrinolytic phenomenon and asserted a possibility of its practical utilization.[282-291] A critical review of experiences, collected with the Fuchs test and published up to 1934, was based on a total of 5000 cases tested, and since correct results appeared in ninety-two to ninety-four per cent, a definite diagnostic value has been assigned to this test.[292] Some sources of error, leading to positive reactions in cancer-free persons, have been disclosed. Such erroneous diagnoses were found to occur commencing with the third month of pregnancy [290] and also in six out of ten cases of gastric or duodenal ulcers.[293] But the main difficulty encroaching upon the practical efficacy of the test is once again—as in many other biological tests—represented by the problem of having available a completely

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[270] H. J. Fuchs: Klin. Wchnschr. *4*: 2350 (1925); Biochem. Ztschr. *170*: 76 (1926); *175*: 185 (1926).

[271] H. J. Fuchs & M. v. Falkenhausen: Biochem. Ztschr. *245*: 304 (1932).

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[272] H. J. Fuchs: Klin. Wchnschr. *12*: 113 (1933).

[273] H. J. Fuchs, M. v. Falkenhausen & W. K. Devrient: Ztschr. f. Immunitätsforsch. u. exper. Ther. *78*: 272 (1933).

[274] H. J. Fuchs & W. K. Devrient: Wien. klin. Wchnschr. *46*: 108 (1933).

[275] H. J. Fuchs: Ztschr. f. d. ges. exper. Med. *98*: 70 (1936); Ztschr. f. Krebsforsch. *44*: 384 (1936).

[276] W. M. Wright & C. G. L. Wolf: J. Cancer Res. *14*: 370 (1930).

[277] B. H. E. Cadness & C. G. L. Wolf: Biochem. Ztschr. *238*: 287 (1931).

[278] G. Montemartini: Bol. ist. sieroterap. milan. *9*: 489 (1930); abstr, Ztschr, f. Krebsforsch, *34*; Ref. *27* (1931),

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[279] K. Yokota: Biochem. Ztschr. *232*: 58 (1931).

[280] F. Friedl & E. Kulka: Zentralbl. f. Gynäk. *58*: 2896 (1934).

[281] H. J. Fuchs: Biochem. Ztschr. *237*: 87 (1931).

[282] M. Bing & G. Marangos. Beitr. z. klin. Chir. *160*: 417 (1934).

[283] K. Hikiji: Tr. Soc. path. jap. *24*: 509 (1934).

[284] V. Kafka, Jr.: Casop. lék. česk. *1*: 750 (1934); Ztschr. f. Krebsforsch. *41*: 369 (1935).

[285] E. Brandt: Klin. Wchnschr. *14*: 1473 (1935).

[286] V. Jedlička & E. Weichherz: Ztschr. f. Krebsforsch. *41*: 148 (1935).

[287] E. Brandt: Ztschr. f. Krebsforsch. *43*: 370 (1936).

[288] F. Bernhard & K. Köhler: Deutsche Ztschr. f. Chir. *248*: 72 (1936).

[289] R. Lothammer & A. Rosenbohm: Ztschr. f. Krebsforsch. *45*: 28 (1937).

[290] R. Lothammer & A. Pistofidis: Ztschr. f. Krebsforsch. *45*: 105 (1937).

[291] D. L. Woodhouse: Lancet *1*: 138 (1937); Am. J. Cancer *40*: 359 (1940).

[292] I. Davidsohn: Am. J. Clin. Path. *6*: 172 (1936).

[293] G. Ruff: Prensa méd. argent. *24*: 671 (1937).

adequate substrate (fibrin or protein) for the performance of the fibrinolytic reaction. Numerous authors stressed the importance of this point, and found themselves frequently unable to prepare a satisfactorily reacting substrate.[291, 294-296] In particular one worker, who encountered the same obstacle in his investigations, concluded from the literature and also from his own experiments that substrates for the test, which yield reaction values definitely exceeding the error-range inherent in the chemical determinations, are only rarely available; moreover, the results obtained were believed to demonstrate a difference between normal and cancer sera only in a statistical sense, but a real diagnostic value in the individual case could not be attributed to them.[297] These circumstances, especially the use of unsuitable substrates, may be responsible for the disappointing diagnostic results recorded in some recent publications on the Fuchs test.[294, 298]

Several modifications of the Fuchs test have been proposed for the purpose of improving its efficiency. Thus the use of serum ultrafiltrates,[284, 299] or of citrate plasma or decalcinized serum [300] was recommended but, according to one study, the latter procedure failed to prove advantageous.[289] Instead of the nitrogen determination devised by Fuchs, methods were proposed in which the protein fission was measured by means of determinations of the free amino and carboxylic groups,[301]

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[294] D. Albers, R. Merten & K. Mucke: Ztschr. f. Krebsforsch. *47*: 512 (1938).

[295] R. Merten & K. Mucke: Ztschr. f. Krebsforsch. *47*: 525 (1938).

[296] C. S. Robinson, R. Evers & A. Truex: Arch. Surg. *41*: 730 (1940).

[297] O. Rosenthal: Am. J. Cancer *37*: 566 (1939).

[298] B. Snellman & B. Ebenius: Klin. Wchnschr. *16*: 464 (1937).

[299] F. Chrometzka & P. Gottlebe: Ztschr. f. d. ges. exper. Med. *86*: 436 (1933).

[300] H. Caspary: Ztschr. f. Immunitätsforsch. u. exper. Ther. *82*: 506 (1934); Klin. Wchnschr. *13*: 668 (1934).

[301] H. Kowarzyk: Bull. internat. Acad. pol. sc., cl. méd. No. 2–5, 67 (1933); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 162 (1935).

or by means of a titration of the basic amino acids liberated from the protein.[302] The latter method was found to be more reliable than the original technique, but nevertheless it likewise depended on the availability of adequate protein substrates.[295] A simplified technique has been recommended which was thought to be particularly adaptable to clinical purposes. It consisted of a conversion of tryptophane, freed by the protein cleavage, to indole by action of Coli bacteria, and the indole was estimated by a color reaction (violet tint) which occurs after the addition of hydrochloric acid and p-dimethylamino-azobenzene.[303] No definite evaluation of the efficiency of this procedure is possible, since it has not been applied to a sufficiently large number of cases. Experiments in which normal fibrin was impregnated with congo red or other stains failed to result in a liberation of the dyes, when subjected to the action of sera of cancer patients.[304]

Other proteolytic enzymes. A close relationship with the fibrinolytic phenomenon is obviously inherent in a diagnostic test based on the observation that normal serum contains enzymes capable of splitting protein of tumor sera, but not that of normal sera.[305] The protein cleavage is determined after incubation by precipitating the serum mixture with alcohol and by adding ninhydrin, a procedure which results in a violet color, due to the presence of low-molecular protein fission products. According to the author and some re-examinations, a score of eighty to eighty-five per cent correct results is accomplished by this test.[305-307] However, especially in tuberculous patients, a high percentage of unspecific reactions were found,[306] and one worker obtained entirely unsatisfactory results in a clinical application of this test.[308]

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[302] H. Minibeck: Ztschr. f. d. ges. exper. Med. *96*: 362 (1935).

[303] J. Čížek: Ztschr. f. Krebsforsch. *42*: 311 (1935).

[304] J. Kabelik: Compt. rend. Soc. de biol. *122*: 421 (1936).

[305] H. Lehmann-Facius & J. Witting: Deutsche med. Wchnschr. *60*: 1714 (1934).

[306] O. Weiss: Wien. klin. Wchnschr. *49*: 493 (1936); *50*: 1416 (1937).

[307] H. Reploh & H. Middeldorf: München. med. Wchnschr. *83*: 1380 (1936).

In the chapter on enzymes, several findings have been discussed which seemed to indicate that the serum peptidases of tumor patients might be differentiated from the normal enzymes, but since none of these studies reached conclusive results, a diagnostic utilization did not appear feasible. The finding that cancer serum contained a peptidase which splits glycyl-tryptophane, whereas this enzyme was not found in normal sera,[309] is of no practical value, since this peptidase was also detected in cachectic conditions of non-malignant origin. Likewise, claims inferring that tumor sera were capable of splitting, in a specific manner, polypeptides containing d-amino acids could not be substantiated by continued work, as was discussed in chapter IV.

Finally, investigations may be mentioned in which enzymes present in the urine and which attack nucleoproteins have been studied. The presence of these nucleoproteases can be demonstrated by adding substrates of organs, prepared by special methods, to the urine and by incubating the mixture. The subsequent addition of acetic acid did not produce a precipitate in tests in which urines of healthy persons were used, because the nucleoproteins of the substrate are split by the action of the urinary enzymes into decomposition products not precipitable by acid. However, a formation of a turbidity after the addition of acetic acid indicates the presence of unchanged nucleoproteins and, correspondingly, the absence of the urinary enzymes. Such a phenomenon is observed in pathologic conditions—not only in neoplasia—when the urine reacts with a substrate derived from the same organ which is pathologically changed in the patient whose

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308. A. Vonessen: Thesis, Kiel 1936; abstr. Ztschr. f. Krebsforsch. *44*: Ref. 148 (1936).

309. R. Schaefer: Deutsches Arch. f. klin. Med. *161*: 313 (1928).

urine is tested, i.e., the absence of specific nucleoproteases in the urine is indicated by this test.[310] The elaboration of these findings, however, has not proceeded far enough to permit a decision as to whether they may represent an adequate basis for a method of cancer diagnosis and whether it may even be possible in this manner to determine the location of the tumor.

Antitryptic test. This reaction was suggested by the theoretical assumption that tumor disintegration and subsequent liberation of tryptic enzymes may induce the formation of antiferments, and that these may be detected in the serum of the patients. In fact, it has been reported that the antitryptic titer was increased in eighty to ninety-five per cent of tumor sera,[311] but it has been stressed that this finding is by no means specific for neoplasia, but is rather due to cachexia, and that it is obtainable also when large amounts of tissue undergo decomposition for other reasons,[312] or in the case of an increased disintegration of leucocytes.[313] Similar results were obtained by means of a modified technique for the determination of the antitrypsin content [314] and, since unspecific positive reactions were frequently seen, only the negative outcome of the test was thought to be a valuable aid to the exclusion of neoplasia.[314–317] Theoretical studies of this phenomenon, performed at a more recent date,[318] have been discussed in chapter IV

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310. E. Freund: Wien. klin. Wchnschr. *46*: 1574 (1933).
311. L. Brieger & J. Trebing: Berlin. klin. Wchnschr. *45*: 1041; 1379; 2260 (1908).
312. E. Waelli: Mitt. a. d. Grenzgeb. d. inn. Med. u. Chir. *25*: 184 (1912).
313. F. Landois: Berlin. klin. Wchnschr. *46*: 440 (1909).
314. E. Peyre: J. Méd. franç. *11*: 472 (1922).
315. Marcus: Berlin. klin. Wchnschr. *45*: 689 (1908).
316. A. Pinkuss: Berlin. klin. Wchnschr. *47*: 2342 (1910).
317. S. de Poggenpohl: Arch. de méd. expér. et d'anat. path. *21*: 657 (1909).
318. W. Raab: Ztschr. f. d. ges. exper. Med. *97*: 588 (1936).

Lipolytic enzymes. The interesting observation has been reported that an atoxyl-resistant lipase is considerably increased, not only in neoplasms (cf. chapter IV), but also in the serum of tumor hosts.[319] It seems plausible to see the source of this enzyme in the tumor, since a rapid decrease of the atoxyl-resistant lipase was found following radical extirpation of the tumor. Although the essential correctness of these findings was confirmed,[320] the diagnostic application does not seem very promising, since—on the one hand—this enzymatic anomaly frequently disappears with advancing tumor growth and—on the other hand—may also be encountered in non-malignant conditions in the case of excessive epithelial regeneration. In a more recent study, positive findings were obtained in approximately two-thirds of 300 cancer cases tested, the best results appearing in skin, mammary and rectal cancers,[288] and therefore this method may only be used as a supporting measure in cancer diagnosis.

Deviations from the normal have been described as prevailing in tumor sera regarding the ability to activate lipatic enzymes. This quality was, however, found diminished not only in neoplasia, but also in many infectious diseases, particularly in tuberculosis,[321-324] and accordingly no practical value can be assigned to determinations of lipase-activators present in serum. In this connection, it may also be recalled that numerous studies, dealing with the kinetics of enzymatic processes, furnished ample evidence that activation of enzymes is not necessarily dependent on definite substances, but may be brought about by a number of chemical and physicochemical factors, generally grouped under the term of "milieu."

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[319] F. Bernhard: Ztschr. f. Krebsforsch. *38*: 450 (1932).

[320] K. Köhler: Ztschr. f. Krebsforsch. *43*: 87 (1936).

[321] J. A. Shaw-Mackenzie: J. Physiol. *41*: 216 (1915); Lancet *2*: 759 (1922).

[322] W. C. M. Lewis: Internat. Conf. Cancer, London, p. 457 (1928).

[323] R. F. Corran & W. C. Lewis: Biochem. J. *22*: 451 (1928).

[324] N. Waterman: Ztschr. f. Krebsforsch. *34*: 313; 327; 334 (1921); *40*: 333 (1931).

Glycolytic enzymes. As has been described in chapter VI, metabolic alterations of seemingly far-reaching significance have been discovered not only in the neoplastic tissues proper, but they have also been found to exist to a certain extent in other organs of tumor hosts. Correspondingly, attempts have been made to devise methods by means of which such metabolic disturbances could be detected in the blood of cancer patients, with a regularity permitting a diagnostic application. In this connection it was reported that red blood cells of cancer patients possess an increased glycolytic activity, as expressed in measurements of the lactic acid formation in vitro, when compared with the behavior of erythrocytes of cancer-free persons.[325, 326] Some authors confirmed this statement,[327, 328] while others were unable to note a characteristic difference between glycolysis of normal or of cancer blood,[329] and hence the practical value of these experiments is inconclusive. Regarding the theoretical background and interpretation of this phenomenon, reference is made to chapter VI, where also another peculiar quality of the blood glycolysis of tumor patients was discussed in detail. This refers to the finding, that the glycolysis of erythrocytes of tumor-free persons is considerably increased in vitro when colloidal solutions of carotene are added, but that blood of cancer patients fails to react in this manner.[330] This difference was

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[325] M. Ascoli & R. Indovina: Klin. Wchnschr. *13*: 956 (1934).

[326] M. Ascoli: Klin. Wchnschr. *14*: 1593 (1935); Compt. rend. Soc. de biol. *119*: 902 (1935).

[327] C. Vercesi & F. Guercio: Riforma med. *50*: 1299 (1934).

[328] D. Campanacci & C. Negri: Libro de Oro, Buenos Aires, p. 198 (1935).

[329] E. J. Sterkin, K. A. Hiada & M. S. Kosarnovskaja: Problems of Oncology (Kharkov) *10*: 44 (1936); abstr. Am. J. Cancer *31*: 127 (1937).

[330] C. Wetzler-Ligeti & R. Willheim: Biochem. Ztschr. *254*: 364 (1932); Wien. klin. Wchnschr. *46*: 1252 (1933).

assumed to be due to the fact that carotene, by virtue of its oxygen avidity, creates quasi-anaerobic conditions, and hence increases the glycolysis of normal blood. Such an effect, however, is not observed in cancer blood. As to the diagnostic usefulness of this test, approximately eighty per cent of correct results were obtained by the original authors,[330] as well as in a recent study.[331] A technical difficulty in the performance of the test is represented by the preparation of suitable carotene solutions which are, by nature, unstable.

A relationship to the enzymatic carbohydrate cleavage may be seen in the finding that—according to some workers—approximately seventy per cent of the urines of cancer patients contain an amylase activator.[332] In spite of the low diagnostic accuracy, this method has been recommended as an expedient test for supporting the clinical diagnosis, since the technique is simple and the material easily accessible; good results obtained with this test were reported in one study.[333]

Oxidative enzymes. Similar theoretical ideas as those mentioned in introduction to the preceding section have been responsible for work of this kind. The activity of blood oxidases is measured in the Sehrt test [334] by means of a colorimetric determination of indophenol blue, formed from naphthol and paraphenylenediamine in the presence of blood. The author found an increase of this color reaction in ninety per cent of cancer patients, as compared with the reaction of blood of normal persons. Increased values found in approximately twelve per cent of seemingly cancer-free cases have been regarded as an indication of a predisposition to neo-

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331. P. Meyer-Heck: Ztschr. f. Krebsforsch. *49*: 142 (1939).
332. P. Geréb: Ztschr. f. Krebsforsch. *36*: 115 (1932).
333. E. Groszwirth: Zentralbl. f. Gynäk. *56*: 2075 (1932).
334. E. Sehrt: Zentralbl. f. Path. *43*: 97 (1928); Virchow's Arch. f Path. *275*: 175 (1929); Ztschr. f. Krebsforsch. *30*: 260 (1929); *37*: 94 (1932); München. med. Wchnschr. *82*: 615 (1935); Klin. Wchnschr. *14*: 19 (1935); Libro de Oro, Buenos Aires, p. 1295 (1935).

plasia. Good diagnostic results derived from this test have been reported,[335] but other authors found the method only partly successful,[336] or even denied its value since correct results were found only in 76.5 per cent of tumor patients, and positive reactions occurred in most anemia cases, though free of malignancy.[337]

A different viewpoint was responsible for investigations dealing with the respiration of hemolyzed blood of cancer patients,[338] namely, these studies were incited by the assumption of a deficiency of the Pasteur effect in the organism of tumor hosts (cf. chapter VI). Actually a different behavior of normal and of cancer blood has been revealed, inasmuch as hemolyzed blood of tumor patients showed an increase of the oxygen consumption when boiled muscle extracts, i.e., oxidative systems, were added, apparently by supplementing a deficient mechanism for oxygen utilization. The oxygen consumption of hemolyzed blood of normal persons, as a rule, failed to be stimulated in the same experimental arrangement in which manometric measurements (Warburg apparatus) were employed. Although the results seemed interesting, not only because of their theoretical implications, but also on account of encouraging diagnostic possibilities, in the latter respect, a definite evaluation cannot be made because of the insufficient experiences which have so far been collected.

3. *Physicochemical Tests.*

Most of the tests to be presented in the following are frequently designated as "lability reactions," and these occupy an exceptional position insofar as they, a priori, do not purport to be specifically connected with the pathology of neoplasia, but in the majority of cases have been devised with a full knowledge of the fact that similar phenomena may also be encountered in other pathologies. Nevertheless, these tests found widespread clinical application because of their generally very simple technique, and perhaps because the expectations associated with them have not been too high to begin with. The principle of lability reactions may be defined as being based on an augmented capacity for precipitation of serum colloids, or, in other words, that the particle size of the colloids is increased. In chapter II such relations have been exemplified by a discussion of the albumin-globulin ratio found in the serum of tumor patients, and it has already been emphasized that similar changes are encountered in a great many pathologies of nonmalignant origin (cachexia, hydremia, kidney affections, liver disorders, etc.). The essential technique of these lability tests consists of producing precipitates in sera by means of more or less simple reagents; but mention will not be omitted of the fact that several authors believed that many tests also of supposedly immunological nature in which the use of biological substrates leads to precipitations (cf. precipitin tests) in reality are merely expressions of a disturbed physicochemical equilibrium manifest in the serum of tumor patients. Furthermore, it appears justifiable to assume that the mentioned changes of the colloid-chemical serum structure may be responsible for the outcome of several tests employing physicochemical methods other than precipitations (Roffo test, meiostagmin reaction, etc.). These tests may be designated as lability reactions in a broader sense.

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[335] W. Michaelis: Zentralbl. f. Gynäk. *59*: 1409 (1935).

[336] V. Kafka: München. med. Wchnschr. *83*: 2126 (1936).

[337] F. O. Höring & H. Schmidt: München. med. Wchnschr. *83*: 2001 (1936).

[338] C. Wetzler-Ligeti & R. Willheim: Ztschr. f. d. ges. exper. Med. *99*: 416; 422 (1936).

Kahn test. The name originally proposed for this test by the author[339] was "albumin-A test," owing to the fact that he separated the serum albumins into three groups: albumin-A, B, and C by means of their capacity for being salted out. Among these, albumin-A is precipitable only by the highest salt concentrations. Incidentally, chemical differences have also been detected in the structure of albumin-A, inasmuch as it was found to be free of tryptophane and rich in histidine.[340] According to Kahn's investigations, albumin-A is lacking or at least considerably reduced in the serum of tumor patients. The original technique of this test consisted of a precipitation of all other serum proteins by means of a 37.15 per cent ammonium sulfate solution, and in testing the filtrate for the presence of albumin-A by heat coagulation. This procedure as a rule has a negative result—absence of a precipitate —in instances of tumor sera. For the purpose of eliminating various sources of error, the author later devised a method by means of which all protein fractions of the serum are determined by employing an optical instrument (opalometer).[341] In this case, too, the absolute and relative diminution of albumin-A is the decisive factor for the diagnosis. Yet from the very beginning the unspecific nature of the test has been stressed, since a similar decrease of the albumin-A concentration in the serum may also be encountered, in addition to neoplasia, in a number of other processes, such as pregnancy, liver insufficiency, kidney diseases, and in all conditions accompanied by a considerable protein deficit. In spite of the fact that with this method several thousands of cases have been tested, the author declined to express the results in the form of statistics, since their aspects would depend decisively on the selection of the tumor and control cases tested. As to the practical value of the test, the author recommended its application primarily for examinations en masse of seemingly

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[339] H. Kahn: Ergebn. d. inn. Med. u. Kinderh. *27*: 365 (1925); Klin. Wchnschr. *3*: 920 (1924); *9*: 262. (1930).

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340. S. Goldschmidt & H. Kahn: Ztschr. f. physiol. Chem. *183*: 19 (1929).

341. H. Kahn: 1st Internat. Cancer Congress, Madrid *2*: 580 (1933).

healthy individuals, who should be then subjected to a thorough clinical check-up in case of a positive test. It is obvious that under these conditions it would be by no means disadvantageous if the positive outcome were to lead to the detection not only of neoplasia, but also of some of the other pathologies mentioned. Numerous re-investigations of the test yielded results corroborating the original findings,[290, 342–353] but some authors arrived at divergent conclusions,[354–358] or denied any practical value to the test, because they found the nephelometric method to be unreliable.[359] It is of interest to refer to a statement that the Kahn test was found highly specific when applied to tumor-bearing rats,[345] a fact which may be due to the relatively simpler conditions prevailing in animals, when compared with human pathology.

Botelho test. The test proposed by Botelho [360] also takes place in the protein fraction of the serum, although the relations in this instance are much less simple than in the

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342. W. Frégonneau: Klin. Wchnschr. *6*: 453 (1927).
343. A. Scarpello: Clin. chir. *3*: 303 (1927); abstr. Ztschr. f. Krebsforsch. *26*: Ref. 45 (1928).
344. B. Wottschall: Ztschr. f. d. ges. exper. Med. *50*: 701 (1926).
345. F. P. Tinozzi: Ztschr. f. Krebsforsch. *24*: 477 (1927).
346. I. Davanzo & G. P. Cucco: Arch. di pat. e clin. med. *8*: 293 (1929); abstr. Ztschr. f. Krebsforsch. *29*: Ref. 105 (1929).
347. H. Giebel: Med. Klin. *25*: 1888 (1929).
348. W. Bachmann: Klin. Wchnschr. *9*: 1540 (1930).
349. A. Haim: Med. Welt *5*: 184 (1931).
350. H. Röhrig: Med. Klin. *28*: 725 (1932).
351. O. Sievers: Acta path. et microbiol. Scandinav. *8*: 29 (1931); *9*: 111 (1932).
352. P. Emiliani: Policlinico (sez. chir.) *42*: 629 (1935).
353. F. Cavazza: Diag. e tec. di lab. *8*: 809 (1937).
354. H. E. Büttner: Klin. Wchnschr. *3*: 1720 (1924).
355. K. Posener & W. Behrens: Klin. Wchnschr. *6*: 853 (1927).
356. M. Fleck: Deutsche Ztschr. f. Chir. *214*: 341 (1929).
357. F. J. Laux: Klin. Wchnschr. *4*: 405 (1925).
358. E. Würth: Thesis, Hamburg 1936; abstr. Ztschr. f. Krebsforsch. *45*: Ref. 249 (1937).
359. K. Hinsberg, R. Merten & K. Mucke: Ztschr. f. Krebsforsch. *49*: 371 (1939).
360. C. Botelho: Bol. Soc. d. med. e cir. Sao Paolo *13*: 315 (1929).

Kahn test, because of the choice of a complex method of precipitation. The serum is first diluted with an 0.75 per cent sodium chloride solution, then a mixture of citric acid and formalin is added, and finally an aqueous iodine solution is admixed; when flocculation occurs after addition of not more than 0.7cc. of the iodine solution, a positive reaction is recorded, whereas precipitation, requiring more than one c. c. of the iodine solution, corresponds to a definitely negative reaction. A modification of the test, employing different concentrations of the reagents, was claimed to increase its efficiency,[361] and by using this method correct results were obtained in seventy-five to eighty per cent of tumor cases, but unspecific reactions were observed in tuberculosis, liver damages, and severe cachexias.[362] These erroneous diagnoses may have been due to hydremia, since it was found that all sera containing less than six per cent protein reacted positively. To eliminate this source of error Botelho recommended always first determining the protein concentration by means of refractometry, and the equalizing of the protein content of the sera prior to the performance of the test. But in spite of this and other proposed modifications,[363] most of the investigators, with few exceptions,[364] arrived at highly unsatisfactory results.[365-374]

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361. A. Wilbouchevitch: Compt. rend. Soc. de biol. *87*: 1339 (1922).
362. F. Faludi: Biochem. Ztschr. *162*: 116 (1925).
363. M. Solecka: Ztschr. f. Krebsforsch. *25*: 314 (1927).
364. T. Calzolari: Policlinico (sez. prat.) *37*: 1549 (1930).
365. A. Cabanis & Ch. Foulquier: Compt. rend. Soc. de biol. *88*: 1011 (1923).
366. B. S. Acevedo: Ztschr. f. Krebsforsch. *28*: 311 (1929).
367. M. Finkelštejn: Vopr. Onkol. *3*: 159 (1930); abstr. Ztschr. f. Krebsforsch. *34*: Ref. 26 (1931).
368. H. Hilarowicz: Ztschr. f. d. ges. exper. Med. *53*: 121 (1926).
369. M. C. Reinhard & K. L. Tucker: J. Cancer Res. *10*: 478 (1926).
370. F. Graziani: Policlinico (sez. prat.) *39*: 637 (1932).
371. F. F. Martinez, E. S. Peregrin & J. M. Clavera: Semana méd. *1*: 1478 (1933); abstr. Am. J. Cancer *20*: 165 (1934).

Bendien test. In contrast to the general remarks presented in the introduction to this section, regarding the efficiency of lability reactions, Bendien [375] claimed that a test devised by him would offer a definite possibility for a specific tumor diagnosis. This test employed precipitation of serum proteins by means of mixtures of vanadic acid and acetic acid. In every test, serum dilutions are treated with varying concentrations of the precipitants, and the results observed are charted in diagrams which, it has been reported, should enable the investigator to differentiate the sera of tumor patients and also of tuberculous persons from the sera of normal individuals. However, re-examinations of this method could not substantiate these claims, inasmuch as the diagnostic efficacy at best equalled that of other lability tests (seventy to seventy-five per cent correct results), [376-385, 291, 374] or even erroneous diagnoses of sixty-six per cent were found in control cases.[386] Likewise, a modification of this test, in which interferometric methods were employed with purport-

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372. G. Rotolo: Atti e mem. soc. lomb. chir. *2*: 603 (1934); abstr. Ztschr. f. Krebsforsch. *41*: Ref. 75 (1935).

373. S. F. Gomes da Costa: abstr. Ztschr. f. Krebsforsch. *43*: Ref. 83 (1935).

374. R. Merten: Ztschr. f. Krebsforsch. *47*: 533 (1938).

375. S. G. T. Bendien: Spezifische Veranderungen des Blutserums, G. Fischer, Jena 1931; 1st Internat. Cancer Congress, Madrid *2*: 457 (1933).

376. E. Meyer: Ztschr. f. Krebsforsch. *35*: 623 (1932).

377. J. R. de Bruine Groeneveldt & L. de Kromme: Nederl. tijdschr. f. geneesk. *75*: 4988 (1931).

378. E. Boyland: Cancer Rev. *7*: 433 (1932).

379. M. Freeman, H. F. Holden, W. W. Lempriere, R. K. Scott, F. E. Williams & C. H. Kellaway: Med. J. Australia *2*: 778 (1931).

380. C. Gordon-Watson: Brit. M. J. *2*: 677 (1931).

381. J. Patterson & L. Adler: Brit. M. J. *2*: 1063 (1933).

382. F. Hogenauer & T. Gröbl: Wien. klin. Wchnschr. *48*: 1320 (1935).

383. E. Tebrich: Thesis, Basel 1935; abstr. Ztschr f. Krebsforsch. *43*: Ref. 133 (1936).

384. O. J. Nielsen: Acta med. Scandinav., Suppl. *78*: 799 (1936).

385. R. E. Jones & D. L. Woodhouse: Quart. J. Med. *5*: 141 (1936).

386. J. Horvath: Monatschr. f. Krebsbekämpf. *6*: 125 (1938).

edly satisfactory success,[387] yielded disappointing findings according to other authors,[381, 388] or was declared to be an entirely unreliable "chance reaction." [389] Nevertheless, a recent report must be mentioned according to which useful prognostic information has been derived from the repeated performance of the Bendien test in 151 cases of cervical cancer, followed-up during eight years.[389a] Additional experience will be required in order to ascertain whether the prognostic value of the Bendien test actually surpasses its diagnostic efficiency.

An obvious relationship to the Bendien test is exhibited by a procedure proposed by Bovenkamp and Kissje,[390] according to whom varying concentrations of sodium chromate and of propionic acid are used for the precipitation of serum proteins. The serum to be tested is employed, both in native and in inactivated form, and the recorded precipitation curves of these two substrates yield a point of intersection which is considered as decisive for the diagnosis of neoplasia. The efficiency of this test corresponded to that of other lability reactions, and was supposed to express a varying adsorbability of electrolytes to serum proteins.

Kopaczewski gel formation test. Kopaczewski [391] studied the gel formation of serum by the action of acids, particularly of lactic acid, and found this phenomenon to be accelerated in eighty-six per cent of cancer sera. The shift of the albumin-globulin ratio and changes of the surface tension, occurring in these sera, were believed to be responsible for the enhanced gel formation and, accordingly, the unspecific nature of the test was admitted. Whereas a few authors found the results of the test, in spite of unspecific reactions in some control cases, as a valuable aid for cancer diagnosis,[392-394] the great majority of investigators concluded that the erroneous positive results in control sera were far too frequent to permit a practical use of the test.[395-404]

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[387] E. Cronin Lowe: Brit. M. J. *2*: 1060 (1932); *1*: 407 (1933); J. State Med. *41*: 530 (1933); abstr. Am. J. Cancer *23*: 612 (1935).

[388] F. C. Smith, E. R. Holiday & J. Marrack: Lancet *2*: 507 (1931).

[389] C. H. G. Price: Brit. M. J. *1*: 551 (1939).

[389a] A. A. Gemmell: Cancer Research *2*: 296 (1942).

[390] G. J. van den Bovenkamp & W. H. Kisjes: Klin. Wchnschr. *13*: 810 (1934); Nederl. tijdschr. f. geneesk. *77*: 2319 (1933); *78*: 2762 (1934).

[391] W. Kopaczewski: Ztschr. f. Krebsforsch. *42*: 262 (1935); Bull. Assoc. franç. p. l'étude du cancer *24*: 551 (1935); Compt. rend. Acad. d. sc. *202*; 990 (1936); Tumori n.s. *10*; 537 (1936).

Sachs test. Whereas the methods discussed so far have been principally concerned with qualities of the serum proteins, in this method an involvement of lipids is apparent. Furthermore, this test has not been developed primarily for diagnostic application, but was rather studied for the purpose of urging a cautious evaluation of the so-called specific immunological reactions. Sachs,[405] in this connection, could demonstrate that by employing entirely unspecific lipids, results are obtainable which closely resemble those of studies in which supposedly specific lipid antigens have been used (cf. immunological tests); namely, by addition of lecithin sols to sera a positive reaction (flocculation) was observed in approximately sixty per cent of cancer sera and in ten per cent of control sera. While this outcome certainly cannot be considered as a basis for a diagnostic test, nevertheless, the

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392. E. Marchese: Fol. ginaec. (Genoa) *32*: 389 (1935); abstr. Ztschr. f. Krebsforsch. *44*: Ref. 71 (1936).
393. N. Toro: Diag. e tec. di lab. *7*: 503 (1936).
394. L. Cappon: Compt. rend. Soc. de biol. *120*: 235 (1935).
395. G. Legrand: Gynécologie *34*: 239 (1935).
396. R. Gillier: Compt. rend. Soc. de biol. *118*: 1324 (1935).
397. M. A. Guy: Bull. Assoc. franç. p. l'étude du cancer *24*: 224 (1935).
398. E. Tantini: Tumori n.s. *10*: 56 (1936).
399. J. G. Basnuevo & R. Sutter: Bol. liga contra el cancer *11*: 259 (1936).
400. E. L. Beluffi & A. Mazzocco: Tumori n.s. *11*: 398 (1937).
401. T. Anardi: Tumori n.s. *12*: 120 (1938).
402. V. di Benedetto & M. Stornello: Acta Cancrol. *2*: 495 (1936).
403. P. Canale: Riv. di clin. med. *36*: 818 (1935).
404. A. Costadoni: Diag. e tec. di lab. *7*: 481 (1936).
405. H. Sachs: Ztschr. f. Krebsforsch. *35*: 275 (1932).

prevalence of positive reactions in sera of tumor patients is striking, and this finding appears significant in conjunction with the results of the "immunological" tests discussed above. Most likely no true antigen-antibody reactions were involved in those reactions, and this was certainly not the case in an older finding of a lecithin flocculation caused by a great percentage of sera of cancer patients tested.[406]

In addition to the tests described so far, time and again various other methods, based on colloid-chemical alterations of the serum in neoplasia, have been tried for their diagnostic efficiency, and reference is made to a survey given on the subject by Woodehouse in 1940.[291] Specific mention may here be made of only a few of these diagnostic tests, which have undergone testing on a broader scale, or which evoke interest on account of their theoretical foundations. Among these may be placed the Brossa test,[407] in which the serum lability is demonstrated by precipitations occurring after additions of congo red and aniline-hydrochloric acid mixtures. A study performed with this method in recent years yielded sixty-five per cent positive reactions in tumor-free patients,[408] and thus demonstrated it as being valueless. A more clearly defined theoretical foundation underlies the "prostaxis reaction" of R. Fischer.[409] The term "prostaxis" has been attached to the phenomenon by which albumin protects globulin against precipitation, and a similar effect may also be observed when gelatin solutions are added to serum; i.e., in this case, the precipitability of serum proteins by alcohol is markedly reduced. If, however, gelatin is added to sera of tumor patients, this "prostaxis effect" fails to take place, or is much less pronounced than when employing sera of tumor-free patients. Experiences with this test were reported to have proved it, though unspecific, to be one of the most efficient for a diagnostic utilization, when compared with other lability reactions. As mentioned in chapter III, the ability of cancer sera to act as protective colloids has been found definitely diminished, as evidenced by experiments with Prussian blue suspensions.[410] Positive findings with this method were obtained in seventy-five per cent of the tested sera of tumor patients, and a diagnostic utilization was held possible in conjunction with other qualities of the serum (Noëls "cancer number," cf. in the following).

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[406] E. Weil & H. Braun: Wien. klin. Wchnschr. *21*: 650 (1908).

[407] G. A. Brossa, G. Bozzolo & E. Lombardi: Minerva med. *6*: 1060 (1926).

[408] L. Bakunin: Semana méd. *1*: 1681 (1937).

[409] R. Fischer: Compt. rend. Soc. de biol. *86*: 124 (1922); *87*: 958 (1922); *88*: 242 (1923); in: J. Kotzareff & R. Fischer: Les cancers et la physicochimie, Vigot frères, Paris 1927, p. 192.

Roffo test. The technique of this test is very simple, and consists of the addition of neutral red to serum in certain volumetric proportions. Sera of tumor-free persons admixed with the stain yielded a yellowish color, whereas a more reddish tint was reported to result in cases of cancer sera.[411] The investigations of the author and his collaborators extended to 22,500 cases up to 1939.[412, 413] The theoretical basis of this phenomenon has not been fully explained; the author and his school believed the reddish color to be the consequence of an increased carbon dioxide content of positively reacting serum, which, in turn, was supposed to be due to increased globulin and lipid concentrations.[414, 415] Other workers found the outcome of the test to be entirely dependent on the albumin-globulin ratio.[416] In this connection the fact was pointed out that beef serum known to be very rich in globulin always yielded positive reactions.[417] On the other hand, a dependence of the neutral red stain on the lipid content seemed proven by experiments which showed that lipid augmentation in serum, accompanying the phenomenon of carcinolysis, was followed by an intensification of the red tint caused by neutral red. A similar color-intensifying effect was observed following the addition of lecithin emulsions or of lipid extracts to serum.[197] Therefore, it was suggested that the Roffo test be viewed as the expression of an increased tissue disintegration in vivo, and of a subsequently occurring accumulation of lipids in the blood. This comparatively unspecific background of the test may explain the fact that its efficiency has met with greatly divergent opinions. The author and his school [412] found the test to be highly specific, erroneous diagnoses being apparent only in 6.4 per cent of tumor-free cases and, in addition, pregnancy was reported not to interfere with the results of the test.[418] The percentage of correct results in tumor hosts varied according to the localization of the neoplasms; that is, sera of skin cancer patients reacted positively only in thirty per cent, whereas internal tumors were accompanied by positive tests in eighty-five per cent of the cases.[419] Results of some investigations corroborated these findings only in part by obtaining sixty to eighty per cent correct results.[420-427] Other research workers announced

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[410] E. L. Lederer: Strahlentherapie *42*: 675 (1931).

[411] A. H. Roffo: Bol. inst. med. exper. para el estud. y trat. d. cáncer *2*: 709; 791 (1925).

[412] A. Gandolfo: Wien. klin. Wchnschr. *45*: 1560 (1932); Bol. inst. med. exper. para el estud. y trat. d. cáncer *7*: 675 (1932); Ztschr. f. Krebsforsch. *37*: 448 (1932); Am. J. Cancer *22*: 363 (1934).

[413] B. Luchetta: Bol. inst. med. exper. para el estud. y trat. d. cáncer *16*: 89 (1939).

[414] L. Correa: Ztschr. f. Krebsforsch. *23*: 112 (1926).

[415] A. H. Roffo: Bol. inst. med. exper. para el estud. y trat. d. cáncer *10*: 1 (1933).

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416. H. Hilarowicz & W. Jankowska-Hilarovicz: Ztschr. f. Krebsforsch. *26*: 214 (1928).

417. O. Bier: Brasil-med. *45*: 52 (1931).

418. L. Correa: Bol. inst. med. exper. para el estud. y trat. d. cáncer *11*: 75 (1934).

419. C. Nuñez: Libro de Oro, Buenos Aires, p. 1833 (1935).

420. A. Astraldi & J. B. Rivarola: Rev. soc. argent. urol. *1*: 169 (1925).

421. F. Pilar & A. Encina: Bol. inst. med. exper. para el estud. y trat. d. cáncer *3*: 401 (1927).

422. J. Thomas: Bol. inst. med. exper. para el estud. y trat. d. cáncer *4*: 611 (1927).

that their experiences with the test indicated more or less of a failure as to diagnostic applicability.[428-439]

Meiostagmin test. A comparatively simple technique, and significant theoretical implications, contributed to the great interest which has accompanied the meiostagmin reaction since it was developed some thirty years ago by Ascoli and Izar.[440, 441] These authors observed initially that interaction of the serum of cancer patients with lipid tumor extracts resulted, after incubation of the mixture, in a lowering of its surface tension. That is, by means of stalagmometry an increase of the number of drops in a certain volume was determined. Such an effect did not take place when sera of tumor-free persons were employed for the test. Methyl alcoholic

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423. S. Cioffari & M. Akkerstein: Tumori n.s. *1*: 371 (1927).

424. B. S. Acevedo: Ztschr. f. Krebsforsch. *28*: 311 (1929).

425. F. Carranza: Bol. inst. med. exper. para el estud. y'trat. d. cáncer *4*: 81 (1928)..

426. J. Bottin: Rev. belge sc. méd. *2*: 749 (1930); abstr. Ztschr. f. Krebsforsch. *34*: Ref. 26 (1931).

427. G. Portmann: Rev. de laryngol. *51*: 12 (1930); abstr. Cancer Rev. *5*: 728 (1930).

428. M. Berić: Ztschr. f. Krebsforsch. *24*: 10 (1927).

429. O. Bajc: Wien. klin. Wchnschr. *40*: 163 (1927).

430. O. U. Lakotkin: Russk. klin. *11*: 654 (1929).

431. R. C. Stoica: Thesis, Cluj (Rumania) 1930; quot. loc. cit. 412 (1934).

432. Pettinari: Clin. y lab. *8*: 199 (1926); quot. loc. cit. 412 (1934).

433. F. Rossi: Clin. chir. *29*: 1030 (1926).

434. V. Carulla Riera: Bol. Cancerol. *1*: 85 (1931); abstr. Ztschr. f. Krebsforsch. *37*: Ref. 126 (1932).

435. F. F. Martinez, E. S. Peregrin & J. M. Clavera: Gac. med. españ. *7*: 79 (1932); abstr. Am. J. Cancer *19*: 880 (1933).

436. E. Borghesan: Valsalva *9*: 684 (1933); abstr. Am. J. Cancer *23*: 142 (1935).

437. A. Althausen, C. Nossalevitch, M. Mirer, M. Languere & A. Zolotarev: Vopr. Onkol. *8*: 57 (1935); abstr. Acta Cancrol. *2*: Ref. 26 (1936).

438. D. Albers: Ztschr. f. Krebsforsch. *47*: 543 (1938).

439. Ch. Anderson & C. Disdier: Arch. Inst. Pasteur Tunis *28*: 269 (1939); abstr. Ztschr. f. Krebsforsch. *50*: Ref. 330 (1940).

440. M. Ascoli: München. med. Wchnschr. *57*: 62 (1910).

441. M. Ascoli & G. Izar: München. med. Wchnschr. *57*: 403; 954; 2129 (1910).

extracts of tumors proved most suitable for the test, but their instability interfered with the expediency of the method. While originally it seemed possible to view the phenomenon described as being of an immunological nature, and to ascribe to the tumor extract the role of an antigen, it soon became evident that such conceptions were unfounded. For it was shown that, instead of tumor extracts, methyl alcoholic pancreas extracts could also be used with similar results [442] and, in continued work, the organ lipids could be successfully replaced by chemically defined substances such as myristylic glyceride,[443] by ricinoleic acid or linoleic acid,[444, 445] or by capronic acid.[446] In other modifications, claimed to be particularly efficient, alcoholic extracts of lecithin,[447] or toluene lecithin extracts [448] were employed. The obviously unspecific nature of these various substrates permits the explanation of the phenomenon in question by assuming that the serum of tumor patients apparently possesses an augmented sensitivity regarding the action of surface-active substances in general. The physicochemical aspect of this property of tumor sera has been discussed in chapter III, and it was there remarked that the "buffering capacity" of tumor sera against the surface activity of added sodium glycocholate [449] or of sodium oleate [450] has definitely been found to be diminished. It has been supposed that this alteration in the physico-chemical structure of cancer sera may depend on a diminished cholesterol content, or on a masking of cholesterol by the increased serum globulins.[451] Similar views have been expressed by Ascoli [452] himself; that is, he envisaged the accumulation of tumor lipids in the blood as being responsible for a blocking of normal serum constituents, which are capable of binding lipids or other surface-active substances. Consequently, addition of such substances to the sera of tumor patients will result only in their loose linkage, and they will be capable of evoking a lowering of the surface tension more easily than in the serum of normal persons. Generally, it may be stated that the ability to "mask" lipids is impaired in the sera of tumor patients.

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[442] F. Micheli & F. Cattoretti: Wien. klin. Wchnschr. *23*: 1555 (1910).

[443] G. Izar & P. Ferro: Biochem. Ztschr. *59*: 234; 236 (1914).

[444] G. Izar: Biochem. Ztschr. *60*: 320 (1914).

[445] A. Castellana: Pathologica *14*: 606 (1915).

[446] H. Grevé: München. med. Wchnschr. *71*: 1466 (1924).

[447] R. Köhler & A. Luger: Wien. klin. Wchnschr. *25*: 1114 (1912).

[448] E. Ferrari & L. Urizio: Wien. klin. Wchnschr. *26*: 624 (1913).

[449] W. Kopaczewski: Compt. rend. Acad. d. sc. *191*: 1155 (1931).

[450] Ch. J. Keller & O. Künzel: Ztschr. f. d. ges. exper. Med. *134*: 286 (1938).

In addition to the stalagmometric method, other techniques also showed a changed response of tumor sera to the addition of "lipid antigens." The complement-fixation test of Izar,[10] the precipitin test of Stammler,[29] and investigations on the anti-hemolytic quality of tumor sera [10] may be recalled here. Furthermore, a biological test has been devised, called "meiostagmin test in vivo," which is based on the fact that the "tumor antigens" mentioned acquired highly toxic qualities when heated to 50° C. When lipids thus treated were mixed with the serum, a different behavior became apparent in accordance with the origin of the serum. When mixed with the serum of tumor patients, the toxic substances are precipitated, whereas they remain in solution when reacting with sera of tumor-free persons. This can be demonstrated by intravenous injection of the precipitate and of the supernatant liquid, respectively, to rabbits, in which the toxic material causes shock and death. This rather complicated and expensive method has been reported to yield favorable results.[453] The original meiostagmin test, and its numerous modifications, have been clinically applied by Ascoli and his collaborators with good diagnostic results, but no absolute specificity could be recognized. Similar observations have been made by the majority of re-investigators. Erroneous diagnoses were found in liver cirrhosis, in Graves' disease, in pneumonia and in jaundice, interestingly also in the sera of patients who had undergone ether or chloroform anesthesia (lipid solvents).[442] The positive outcome of the test was not considered as connected with cachexia. On the contrary, negative results were obtained with the sera of severely cachectic patients afflicted with generalized carcinosis.[454] On the other hand, the meiostagmin test was claimed to be suitable for the early diagnosis of neoplasia.[455] The efficiency of the test is sufficiently great to permit its practical application according to a great many authors.[454, 456–466] However, from a number of studies an interesting fact may be inferred by comparing the diagnostic results. It becomes apparent that those workers, who reported on a high number of correct diagnoses in tumor patients (eighty-six to ninety per cent), recorded a comparatively high percentage (up to twenty per cent) of erroneous diagnoses in control cases.[451, 455, 467] Vice-versa, a low quota of correct cancer diagnoses was accompanied by good agreement

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[451] W. Weis-Ostborn & O. Ehrenteil: Biochem. Ztschr. *148*: 308 (1924).

[452] M. Ascoli: Ergebn. d. inn. Med. u. Kinderh. *25*: 944 (1923).

[453] G. Izar: München. med. Wchnschr. *58*: 1347 (1911); Berlin. klin. Wchnschr. *48*: 1748 (1911).

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454. N. Waterman: Ztschr. f. d. ges. exper. Med. *37*: 249 (1923).
455. M. Rosenberg: Deutsche med. Wchnschr. *39*: 926 (1913).
456. F. Cattoretti: Wien. klin. Wchnschr. *24*: 637 (1913).
457. A. Bertinq: Ginecologia *7*: 673 (1910).
458. S. d'Este: Berlin. klin. Wchnschr. *47*: 897 (1910).
459. F. Micheli & F. Cattoretti: München. med. Wchnschr. *57*: 1122 (1910).
460. P. de Agostini: Biochim. e terap. sper. *2*: 125 (1910).
461. C. Stabilini: Berlin. klin. Wchnschr. *47*: 149 (1910).
462. F. Tedesko: Wien. med. Wchnschr. *60*: 1514 (1910).
463. G. Wolfsohn: Arch. f. klin. Chir. *102*: 247 (1913).
464. J. Halpern: Mitt. a. d. Grenzgeb. d. inn. Med. u. Chir. *27*: 340 (1913).
465. F. Rossi: Clin. chir. *10*: 1030 (1926); abstr. Cancer Rev. *3*: 221 (1928).
466. L. Schneider: Am. J. Obst. & Gynec. *21*: 340 (1931).
467. J. R. von Zubrzycki: Arch. f. Gynäk. *102*: 152 (1914).

of the test with the clinical diagnoses in tumor-free persons (more than ninety per cent correct results).[468-471] Most probably these findings depended on the specific qualities of the lipid substrates employed.

Finally, it may be remarked that Ascoli and his co-workers[472] in recent years abandoned the use of the original meiostagmin test in favor of other methods, aiming at the demonstration of physicochemical alterations in the sera of tumor patients. These investigations led to the conclusion that the chief characteristics of the serum of tumor patients —and perhaps also of the whole body of the tumor host—are represented by an increased presence of unsaturated, disposable valences. This fact should become apparent from the following tests: firstly, a cold ether extract of cancer sera exhibits a raised iodine number;[473] according to a later study the values in normal persons averaged 0.27, but were increased in diabetes, liver and kidney disorders, and 18 out of 19 tumor cases showed values exceeding 0.5.[474] Two, ricinoleic acid, when added to cancer serum, can be re-extracted, but not when added to normal sera; i.e., the sera of cancer patients are lacking the ability to bind firmly this fatty acid, a phenomenon which was also utilized for a precipitin test.[31] Three, bilirubin, added in vitro, can be re-extracted from cancer sera, but not from normal sera.[475-478] Four, as mentioned above, the presence of unsaturated compounds was believed

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468. K. Hara: Deutsche med. Wchnschr. *40*: 1258 (1914).
469. L. Arzt & S. Zarzycki: Wien. klin. Wchnschr. *27*: 227 (1914).
470. R. Köhler: Wien. klin. Wchnschr. *31*: 1007 (1918).
471. F. Leidi: Berlin. klin. Wchnschr. *48*: 1716 (1911).
472. M. Ascoli: Klin. Wchnschr. *14*: 1593 (1935).
473. C. Vercesi & F. Guercio: Biochem. Ztschr. *279*: 229 (1935).
474. G. Sorge & A. Baserga: Bol. soc. med.-chir. Catania *4*: 60 (1936); abstr. Ztschr. f. Krebsforsch. *44*: Ref. 202 (1936).
475. M. Ascoli & R. Indovina: Biochim. e terap. sper. *21*: 99 (1934).
476. M. Ascoli: Biochim. e terap. sper. *21*: 343 (1934).
477. M. Ascoli, d'Alessandro & Indovina: Klin. Wchnschr. *13*: 703 (1934).
478. G. d'Alessandro & R. Indovina: Biochim. e terap. sper. *22*: 1 (1935).

to be responsible for an increased glycolysis of red blood cells of cancer patients.[479] By simultaneously applying all these reactions, reliable diagnostic findings were reported to be obtainable,[427, 480–483] whereas other authors found them less useful, especially in early stages of the tumor process.[484] A corroboration of the "bilirubin test" has been furnished by a study, according to which sera of icteric patients also permit a differentiation according to the pathology present; bilirubin could not be extracted from the sera of eighteen jaundiced patients, clinically proven to be cancer-free, whereas the pigment was extractable from twenty out of twenty-two established cancer cases with icterus.[485]

4. *Determinations of Chemical and Physicochemical Constants.*

There was ample opportunity in the first three chapters of this book to refer to endeavors to detect chemical deviations from the normal in the blood or serum of cancer patients, and to utilize these findings for diagnostic purposes. However, none of these efforts appear to have accomplished more than very doubtful results, and only a limited value can be assigned to most diagnostic procedures employing methods of analytical or physical chemistry enumerated in the following.

Methods of analytical chemistry. A method employing analytical determinations of inorganic serum constituents is represented by the Links test.[486] The theoretical basis of this diagnostic reaction was furnished by the claim of the author that serum portions, derived from the blood clot, exhibit variations of their inorganic constituents—calcium, potassium, magnesium—when the serum is separated from the clot at an earlier or later time after the blood coagulation. Accordingly, the author determined the potassium and magnesium concentrations of these serum portions, obtained from the same blood at certain intervals, and arranged the values thus determined in an empirically chosen equation, the result of which was to furnish a characteristic "cancer number," decisive for the diagnostic evaluation. However, these results of exact analytical chemistry are almost forced into the background by corrective factors of a clinical nature (age of the patient, number of erythrocytes, various data of the case history) which the author found necessary to apply to the "cancer number" in the way of subtractions or additions (twenty to fifty per cent). This definitely unscientific approach is reflected in the fact that the astoundingly favorable results of cancer diagnostics reported in publications based on the work of the author himself [487, 488] were soon completely contradicted by other workers, who either submitted "blind tests" to Links [489] or carried out their investigations independently.[490] Furthermore, the theoretical premise of the test, i.e., the electrolyte shifts in serum portions separated from the blood clot at different times, has been only partly confirmed,[491] or has been entirely rejected on the basis of analytical work.[492, 493] Consequently, any prac-

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[479] R. Indovina & W. Fabisch: Ztschr. f. Krebsforsch. *42*: 63 (1935).

[480] E. Ponzi: abstr. Ztschr. f. Krebsforsch. *42*: Ref. 81 (1935).

[481] S. Maugeri: abstr. Ztschr. f. Krebsforsch. *42*: Ref. 129 (1935).

[482] A. Greco: Biochim. e terap. sper. *24*: 191 (1937).

[483] S. Fiandacca: Biochim. e terap. sper. *21*: 518 (1934).

[484] E. L. Beluffi & A. Mazzocco: Riforma med. *1*: 459 (1936); abstr. Ztschr. f. Krebsforsch. *44*: Ref. 199 (1936).

[485] D. Albers & R. Merten: Ztschr. f. Krebsforsch. *49*: 375 (1939).

[486] R. Links: Med. Klin. *30*: 165 (1934); Ztschr. f. Krebsforsch. *41*: 166 (1935).

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487. L. Schönbauer: 1st Internat. Cancer Congress, Madrid *2*: 601 (1933).
488. H. Weber & F. Schüle: Chirurg *6*: 633; 765 (1934).
489. R. R. von Oppolzer & R. Scholl: Chirurg *7*: 412 (1935).
490. E. Epstein: Wien. klin. Wchnschr. *48*: 1179 (1935).
491. H. Waelsch: Wien. klin. Wchnschr. *48*: 480 (1935); Med. Klin. *31*: 451 (1935).
492. P. Fantl: Wien. klin. Wchnschr. *50*: 1486 (1937).
493. H. Thelen: Ztschr. f. physiol. Chem. *246*: 194 (1937).

tical or theoretical value must be denied as being inherent in the Links test.

As to organic blood constituents, a test recommended by Chodat[cf. 494] consisted of adding tyrosinase and para-cresol to serum, and this procedure was claimed to result in a brown color when sera of tumor patients were employed. This phenomenon was thought to depend on an increased content of amino acids in the serum but, in accordance with the doubtful theoretical basis, the practical results obviously failed to establish a usefulness of this test which is now obsolete.

Deviations of the carbohydrate chemistry of blood, supposed to occur in neoplasia, have been discussed in chapter II, especially with regard to the fraction of proteid sugar, but it was stressed that augmented concentrations of this substance discovered in the blood of tumor hosts cannot be regarded as being specific, and hence as not applicable for diagnostic purposes. A similar attitude must be taken towards findings which indicated abnormal shapes of the curves derived from blood sugar tolerance tests in tumor patients (higher peak and delayed return to the initial level).

Determinations of blood lipids have formed the basis for the Noël test;[495] the values of the "acid number" and of the "saponification number" of an ether extract of the serum to be tested were determined and arranged into an empirical formula. In the case of sera of tumor patients, the figure obtained was claimed to exceed the values recorded in normal cases. Furthermore, characteristic quantitative relations between the cholesterol content and the total fat in the blood have been used by the author for a diagnostic evaluation and, in addition to these tests, the final diagnosis should be supported by the measurement of the colloid-pro-

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[494] R. Chodat & R. H. Rummer: Biochem. Ztschr. *65*: 392 (1914).

[495] L. v. Noël: Strahlentherapie *42*: 616 (1931).

tecting action of serum [410] and by spectrometric demonstration of lactic acid and fumaric acid in the blood.[496] Several objections of a theoretical nature have been raised against this combined diagnostic method, and practical examinations could not prove a usefulness of the results thus obtained.[497–499]

An alteration of the blood-coagulating mechanism was claimed to be present in neoplasia, and it led to the development of the Mendeleéff test.[500] The principle of this method was described as the fact that heparinized blood plasma of tumor-free persons is brought to clotting when extracts of leucocytes are added, but coagulation fails to occur when extracts of erythrocytes are used; in neoplasia, the reverse effects were seen in heparinized plasma. A diagnostic efficiency of ninety-five per cent was claimed by the author of the test, but absolutely inconclusive results were found by another worker employing this method.[501]

Physicochemical analyses. Brief reference only is made to those investigations which have been described in chapter III, and which attempted diagnostically to utilize determinations of the hydrogen ion concentration or of the surface tension in the blood or serum of patients with neoplasia; for, no conclusive evidence could be furnished that any of these measures could be useful for practical purposes. Nor does a definite evaluation seem possible with regard to a diagnostic test in which electrometric determinations of the "residual tension" of alcohol-ether-benzene extracts of serum were performed. In the case of tumor sera, characteristic

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496. F. Dannmeyer, O. Hartleb & J. Schubert: Strahlentherapie *42*: 648 (1931).

497. Bennhold; Griesbach: Klin. Wchnschr. *11*: 1523 (1932).

498. L. Brauer, F. Goos & C. Heller: Strahlentherapie *47*: 599 (1933).

499. A. Woodward: Biochem. J. *30*: 1463 (1936).

500. P. Mendeléeff: Bull. Assoc. franç. p. l'étude du cancer *27*: 807 (1938); Cancer, Bruxelles *11*: 135 (1934); Compt. rend. Soc. de biol. *123*: 135 (1936).

501. E. Salamon: Ann. Inst. Pasteur *57*: 299 (1936).

differences from normal values were reported to become apparent as the result of such measurements; eighty per cent correct results were claimed, and a connection of this alteration with the phospholipid metabolism in neoplasia was assumed.[502] Yet, to the knowledge of the writers, no publication repeating and corroborating these experiments has appeared.

A much sounder theoretical basis and much more extensive elaboration can be reported of the polarographic method, which represents an established physicochemical procedure used for various analytical purposes. Its principle may be explained as an electrolytic reduction of chemical substances by means of a "dropping" mercury cathode, for which every chemical compound requires a characteristic voltage. Brdička [503] was the first to propose and use this method for cancer diagnosis, and he reported on interesting findings obtained with sera of tumor patients, which permitted their differentiation from the sera of normal persons. Preliminary investigations corroborated these statements,[504-508] but at the same time it became clear that the test was not specific enough to warrant its clinical application since, in addition to neoplasia, several other conditions (hepatic disorders, pregnancy, febrile infections, circulatory decompensation) also led to similar changes of the polarographic values and, on the other hand, early stages of the tumor process could not be

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502. F. Dannmeyer, J. Schubert, L. Treplin & E. Schnohr: Acta path. et microbiol. Scandinav. *14*: 24 (1937).

503. R. Brdička: Nature, London *139*: 330; 1020 (1937); Compt. rend. Soc. de biol. *128*: 54 (1938).

504. F. Bergh, O. M. Henriques & J. Schousboe: Nature, London *141*: 751 (1938).

505. J. Heirovský: Nature, London *142*: 317 (1938).

506. R. K. Felkel: Med. Klin. *34*: 840 (1938).

507. E. Waldschmidt-Leitz: Angew. Chemie 1938: 324.

508. E. Waldschmidt-Leitz & K. Mayer: Ztschr. f. physiol. Chem. *261*: 1 (1939).

verified by the test.[509-516] As to the theoretical explanation of the different reactivity of cancer sera, established in the majority of the cases, it was assumed that this may depend on a diminution of sulfhydryl compounds in the sera ([509], cf. chapter II), but the validity of this view has been doubted on the basis of pertinent experiments.[517] A clarification of this problem may be expected from continued work, as well as an answer to the question as to whether technical modifications might increase the diagnostic efficiency of polarography.

5. *Urine Tests.*

Although several references have been made in this, or in other chapters, to attempts at employing urine analyses for diagnostic purposes, the subject may be supplemented here by discussing a number of tests in which this substrate has been used exclusively. One of the oldest methods of this kind is the Davis test,[518] consisting of treatment with hydrochloric acid of the residue of an ether extract of urine: the appearance of a red color indicates a positive result. Reinvestigations, however, demonstrated the test to be strongly influenced by the diet used by the patient,[519, 520] and whereas one worker was inclined to attribute a greater diagnostic

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509. A. C. Walker & S. P. Reimann: Am. J. Cancer *37*: 585 (1939).
510. H. E. Wedemeyer & Th. Danz: Ztschr. f. Krebsforsch. *49*: 10 (1939).
511. E. Poli: Diag. e tec. di lab. *10*: 565 (1939).
512. P. Meyer-Heck: Ztschr. f. Krebsforsch. *49*: 560 (1939).
513. H. W. Schmidt: Ztschr. f. Krebsforsch. *50*: 390 (1940).
514. E. Chytrek: Deutsche med. Wchnschr. *66*: 190 (1940).
515. D. Albers: Biochem. Ztschr. *306*: 236 (1940).
516. A. Langer: Science *93*: Suppl. No. 2417, p. 8 (1941).
517. C. G. Wolffbrandt: Nature, London *142*: 212 (1938).
518. T. G. Davis: Am. J. M. Sc. *145*: 851 (1913); Berlin. klin. Wchnschr. *50*: 1312 (1913).
519. F. P. Tinozzi: Diag. e tec. di lab. *2*: 121 (1930).
520. S. Jagunow: Zentralbl. f. Gynäk. *54*: 1954 (1930).

significance to the negative outcome of the test than to the positive one,[521] other studies found it to be of little reliability in both directions,[522, 523] or rejected it completely.[524, 525] Little is known of the chemical background of this test, and the same applies to two other urinary tests proposed. In one of them the urine is acidified with nitric acid, a precipitate is produced with a bromide solution, which is then dissolved in ammonia and treated with benzaldehyde; a yellow to brownish color was interpreted as a positive reaction.[526] Another method consisted of a treatment of urine with ferric chloride and hydrochloric acid, and subsequent extraction of the urine with amyl alcohol; a reddish-brown color of the extract was observed in two-thirds of the urines derived from patients with gastric cancer, and also in some other diseases, but not in the urines of patients suffering from extra-gastric tumors.[527] It seems plausible to assume that most of these tests depend on the urinary excretion of products of intestinal protein putrefaction, related to indoxyl. An interesting report has been made regarding the fluorescence of urines of tumor patients; an increase of this property observed in cancer patients was not regarded as being of specific nature, but a strong diminution of the urinary fluorescence following ether extraction of the urine was thought perhaps to represent a characteristic quality of urines in neoplasia, since this phenomenon was found in ninety-four per cent of proven cancer cases.[528] Although certainly in need of further corroboration, these findings must be regarded as significant in connection with phenomena of urinary fluorescence detected in animals treated with carcinogenic hydrocarbons (cf. chapter II).

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[521] W. E. Jolkwer & W. J. Matschan: Arch. f. klin. Chir. *146*: 593 (1927).

[522] J. Lipkin: Moskowskij med. J. *7*: 8 (1927).

[523] L. Megnani: Riv. ital. di ginec. *8*: 474 (1929).

[524] L. Sanguigno: Diag. e tec. di lab. *3*: 193 (1932).

[525] G. Mariconda: Arch. ital. di dermat. i sifil. *10*: 68 (1934).

[526] L. C. A. Haffner & W. H. Bueermann: West. J. Surg. *39*: 741 (1931).

[527] E. Fischbach: Monatschr. f. Krebsbekämpf. *6*: 44 (1938).

[528] F. I. Simon: Acta med. Scandinav. *100*: 553 (1939).

A test which apparently exhibits a relationship to endocrinology has been devised and recommended by Aron.[529, 530] He reported that the urines of cancer patients contain a substance, precipitable by alcohol, which injected into rabbits or guinea pigs causes a marked lipid loss of the fascicular zone in the adrenals of the animals, a phenomenon claimed by the author to be highly specific for neoplasia, though technically very complicated. Of re-examinations, however, only few yielded satisfactory results,[531] whereas many workers found the test entirely unreliable [532–535] and suspected positive reactions to be due to an increased polypeptide content in the urines tested,[535] an occurrence which has been shown in chapter II to be only a secondary effect of neoplasia. In later experiments, Aron developed another diagnostic test, according to which mixtures of urine and of a serum, both derived from tumor patients, showed, after incubation, a precipitation which was supposed to be a characteristic phenomenon, but it was admitted that this method was not as yet applicable for practical use.[536] Actually a re-examina-

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529. M. Aron: Presse méd. *42*: 833 (1934); Compt. rend. Soc. de biol. *121*; 973; *123*: 246; 248 (1936); *124*: 370; 373 (1937).

530. A. Stoltz, M. Aron, A. G. Weiss & J. Kuntzmann: Presse méd. *44*: 561 (1936).

531. H. Takatsuki: Ztschr. d. jap. chir. Gesellsch. *36*: 134 (1935); abstr. Ztschr. f. Krebsforsch. *45*: Ref. 43 (1936).

532. P. Tagariello: Riforma med. *52*: 246 (1936).

533. G. Roussy, Ch. Oberling & P. Guerin: Presse méd. *42*: 1641 (1934).

534. E. Barnabeo: Bull. d. sc. méd. *107*: 265 (1935); abstr. Am. J. Cancer *21*: 310 (1937).

535. H. Bulliard & I. Grundland: Compt. rend. Soc. de biol. *128*: 298; 302; 999 (1938).

536. M. Aron: Compt. rend. Soc. de biol. *124*: 340; 373 (1937); *131*: 550 (1939).

tion of this method yielded very unfavorable results (positive in sixty-three per cent of cancer patients, in fifty per cent of luetics, and in thirty-five per cent of acute and subacute infections).[537]

Great expectations have been aroused in recent times by the announcement that the urines of tumor patients contain substances which injected into pregnant rabbits caused abortion, and that this property was highly specific for the urines of persons afflicted with malignancy.[538] However, several studies on this phenomenon seemed incapable of proving a specific nature of these findings for neoplasia. Of ten cancer cases analyzed in one study, eight exhibited abortifacient agents in urine and serum, one in urine only, and one in neither urine nor serum, but since in six control cases the serum contained similar substances in three instances, and the urine in two, the author concluded that the toxic substances causing abortion could not be regarded as being specific for cancer.[539] This view seems substantiated by another investigation in which negative results, as to abortifacient action, were obtained with the urines of four cancer patients (one mammary cancer, two skin epitheliomas and one metastasized cancer of the mouth).[540] Likewise factors concentrated from the urines of advanced cancer cases, and which caused fetus resorption in pregnant mice and rats, could also be demonstrated to be present—though in lower concentrations—in the urines of healthy persons.[541] Protein-like precipitates were prepared from the urines of twenty-five cancer patients, and proved in every instance abortifacient for pregnant mice, but a similar effect was observed in seven out of thirty-three urines of cancer-free persons, when analogous precipitates were injected into pregnant mice.[542] Another author could also not obtain results proving a specific occurrence of abortifacient substances in neoplasia.[543] An investigation of recent date showed that substances, abortifacient for pregnant mice, could be prepared from all of seven tested cancer urines, but also from one urine out of four tested and derived from healthy persons, and from three urines of diseased, but cancer-free women. It is noteworthy that the active substances were present in the latter urines in the same concentrations as in the urines of cancer patients, and that the abortifacient action of urinary substances could not be assumed to be identical with a similar effect of prolactin or of peptone, since these substances were found to require higher doses for causing abortion than the urinary factors did.[544] As far as the experimental data available at present permit a definite evaluation, it seems highly probable that the abortifacient action of urinary factors, found in cancer patients or in cancer-free persons, can be believed to be due to the presence of toxic substances, most likely of protein breakdown products, the increased urinary excretion of which, in neoplasia, has been discussed in detail in chapter II, and which, as stated, has been recognized to be not specific for malignancy. Accordingly, a diagnostic usefulness of this test is—at least at present—doubtful.

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[537] C. Scaglioni: Pathologica *31*: 8 (1939).

[538] Th. H. Elsasser & G. B. Wallace: Science *89*: 250 (1939).

[539] K. W. Thompson, Th. Hale, Jr. & B. B. Whitcomb: Am. J. Cancer *37*: 233 (1939).

[540] J. Gershon-Cohen, M. Samson & H. Shay: Am. J. Cancer *38*: 109 (1940).

[541] J. O. Ely: Am. J. Cancer *39*: 379 (1940).

6. *Combinative Methods.*

In view of the fact, demonstrated so convincingly in the preceding parts of this chapter, that not one of the numerous diagnostic tests for malignancy actually proved satisfactory, the tendency to employ a somewhat different approach to tumor diagnostics has gained momentum in recent years. That is, several attempts have been made to attain a safer guidance on the road to clinical diagnosis by simultaneously performing a number of cancer reactions which have been shown to be to a certain extent reliable. Thus, it was reasoned, it might be feasible to exclude sources of error inherent in the individual test, and to construct a composite picture, furnishing more valuable criteria of diagnostic significance. Some methods, described above, belong to this category of cancer diagnosis. The latest work of Ascoli was not confined to the original meiostagmin test, but also made use of a number of other qualities of blood and serum, believed to be characteristic for neoplasia, and Noël's "cancer number" was based on several determinations within the range of blood lipid chemistry. Recently, Freund and his school [545] have also proposed to supplement the performance of the carcinolytic test with the following additional investigations. Examination of the intestinal flora by bacteriological methods and by testing colonies of intestinal bacteria for the presence of lytic or antilytic factors; performance of the Freund-Kaminer skin test; and determinations of the proteid sugar in the blood and of the colloidal nitrogen excretion in the urine. Some workers reported they had obtained remarkably favorable results by adopting this procedure; [546, 547] however, another author found these combined diagnostics even less efficient than the carcinolytic test alone.[548] In one investigation, in which the tests of Abderhalden, of Bendien, of Freund and Kaminer, and of Fuchs,

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[542] E. Klar: Ztschr. f. Krebsforsch. *50*: 155 (1940).

[543] G. Amabile: Rass. ostet. *49*: 180 (1940); abstr. Ztschr. f. Krebsforsch. *51*: Ref. 152 (1941).

[544] M. Ross & R. I. Dorfman: Cancer Research *1*: 158 (1941).

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545. E. Freund: Oberösterreich. Ärztetag., Linz, April 1935.
546. B. Benda & J. Kretz: Wien. klin. Wchnschr. *49*: 174 (1936).
547. J. Kretz: Med. Klin. *33*: 1149 (1937); Carcinom, Wissenschaftl. Verl.-Buchhandlung f. Medizin, Vienna 1938.
548. R. Scholl: Wien. klin. Wchnschr. *51*: 298 (1938).

were used, it was concluded that this arrangement failed to yield results of the desired reliability.[549] Other authors reported on a diagnostic reliability of eighty-five per cent, by applying simultaneously the following methods: The Ascoli test, determination of atoxyl-resistant lipase, Freund-Kaminer test, Fuchs test and polarography,[550] and good results, though not detailed statistically, were obtained by the combined use of the following five reactions: Fuchs test, polarography, Lehmann-Facius test, Freund-Kaminer test, and determination of the atoxyl-resistant lipase.[551] Undoubtedly much more extensive experiences will be required before it can be determined whether continued efforts in this direction may provide a solution to the problem of a diagnosis of neoplasia valuable in practice.

Summary.

There is no need for reiterating here the statement that the subject of this chapter, a reliable and reproducible biological or chemical method of tumor diagnosis, has as yet not found realization, but in connection with this apparent shortcoming of the diagnostic branch of cancer research, it is deemed appropriate to expand on some fundamental questions, the answers to which may considerably change the outlook of those aiming at devising tests for cancer diagnosis. For this purpose we wish to proceed from the hypothetical assumption that there already is at our disposal a method of biological, serological or chemical nature enabling us to diagnose neoplasia in a reliable and specific manner. In what respect, and to what extent would this accomplishment contribute in the fight against cancer in general, and how would it determine the course of medical action in the individual case? Brief consideration will make one cognizant of the fact that even the definite establishment of the presence of neoplasia in a patient cannot be regarded as a decisive factor in the medical management of this patient, so long as at least one of the following two prerequisites has not been provided. One, a means of diagnosing by a generally applicable method not only the presence of a malignant tumor, but also the determining of its exact localization in the body, particularly in those numerous cases in which unclear clinical symptoms fail to give clues for the location of the suspected neoplasm; two, an internal cancer therapy which would make it feasible effectively to attack neoplastic processes located anywhere in the body, in contrast to the therapeutic measures of proven usefulness available now, which—in the form of surgery or radiation therapy—obviously represent local treatments, and consequently necessitate the previous exact localization of the lesion to be treated. Since neither of these two presuppositions—localizing tumor diagnostics, or general cancer therapy—have so far been realized, the value of cancer-diagnostic measures, even if they attain an efficiency of a hitherto unknown degree, is by necessity severely curtailed. For this and for other reasons, the writers of the book look with a certain reserve upon the demand, frequently encountered in the literature, to establish diagnostic procedures which, in particular, could serve as a reliable means of an early tumor diagnosis, i.e., revealing neoplasia at a time when clinical symptoms are either lacking or very obscure. Apart from the great difficulty of achieving this goal, impressively illustrated by the futile efforts of hundreds of workers of the past, we cannot share the anticipatory enthusiasm of those who would regard such an accomplishment as a milestone on the road to the ultimate aim in the fight against cancer. For, even during the course of a very limited practical activity, the writers have more than once had to face the painful situation arising from the fact that biochemical and serological methods definitely indicated the presence of neoplasia in a certain patient, but that this discovery failed to change the predestined course of the disease because, at this time, the available clinical methods were incapable of determining the site of the occult internal tumor, and, at a time when these efforts succeeded, the malignancy had already advanced too far for effective therapeutic measures, such as are known today. There is no doubt that similar experiences have been collected in great numbers by all those engaged in clinical management of malignant diseases. Moreover, the circumstance that it is absolutely necessary for purposes of lay propaganda to stress the great importance of, and the benefits derived from an early tumor diagnosis should not conceal the regrettable fact that, in a great many neoplastic conditions, the ultimate outcome cannot be decisively affected by recognizing the malady even at the earliest time; that is, in these cases, any therapeutic measure now available fails to counteract the tumor process effectively. This inability to utilize even reliable diagnostic findings for rational medical action is still more pronounced in those instances where diagnostic tests, as described in the foregoing, have been interpreted as indicating a predisposition of the patient to neoplasia. Whereas diagnostic findings in other pathologies—e.g., in the form of a positive Wassermann reaction in latent lues, or in the form of skin tests, indicating an abnormal susceptibility of an individual to certain infectious diseases—will initiate sound medical action, no such possibility is at present available when a disposition to cancer is detected.

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[549] P. N. Panton: Lancet *1*: 793 (1937).

[550] H. Griessmann, K. Köhler & W. Söhnel: Chirurg *10*: 609 (1938).

[551] F. Bernhard: Deutsche med. Wchnschr. *65*: 596 (1939).

While the preceding remarks were intended to outline the present-day limitations of even highly reliable laboratory diagnostics of malignancy, the following deliberations are aimed at preventing the reader from falling into the other extreme, that is, of condemning endeavors in the field of tumor diagnostics as being valueless and doomed to failure. Such a view does not appear justified in the writers' opinion, both when the work of the past is taken into consideration and when prospects of continued research are considered, especially when undertaken in a planned manner and with a full awareness of their limits of efficiency. First of all, the significance of some phenomena, discovered in the course of diagnostic investigations, should not be underrated, in spite of the fact that the practical results have not achieved such a reliability as is required for practical purposes. This refers to the carcinolytic reaction, the fibrinolytic reaction, and some other enzymatic processes found to deviate from the normal in neoplasia. The greatest obstacle in the way to a practical utilization of these theoretically significant findings seems to lie in the difficulty of obtaining suitable substrates for the performance of these tests, a circumstance which is generally encountered when biological material of unstable and insufficiently defined nature has to be used. In this regard, future work will decide whether it may be possible, as has already been attempted, to replace the substrates employed up to now by others which are more clearly defined, and the preparation of which is regularly reproducible. As far as immunological tests, which also require biological substrates in the form of antigens are concerned, the prospects seem at present less encouraging, principally because of the lacking or contradictory theoretical foundation, but this view may be subject to revision, as and when necessitated by the presentation of new findings. An entirely different aspect is represented by the lability reactions, since these tests, a priori, are recognized as not being specific for neoplasia but, instead, as indicating disturbances of the physicochemical serum structure which may also occur in the course of other pathologies. But, in addition to their generally simple technique, these tests may be employed with advantage for general health examinations, undertaken periodically. Consequently, a negative outcome of these tests may be valuable by excluding a number of dreaded diseases whereas positive results will give occasion for more specialized diagnostic procedures. Finally, work of recent times has made available some stepping stones to a new approach, in the form of chemical or physicochemical findings facilitating a diagnostic differentiation of neoplasms, at least in later stages of the disease. In this connection particular reference is made to the precise method of polarography.

To the writers' mind, the most promising aspects may be ascribed to efforts of comparatively recent date which employ a combination of several diagnostic tests. Although the evidence presented so far is much too meager to warrant definite conclusions, it is believed that a number of factors may be regarded as being in favor of such procedures. For one, it may be expected that sources of error inherent in particular tests can more easily be rendered innocuous when other tests serve as controls. Naturally, only large-scale experiences will show which of these conflicting results is deserving of greater validity in an individual case. Furthermore, it has been shown that certain types of tumors more frequently yield positive reactions with one method than with others and, likewise, the erroneous diagnoses furnished by various tests in non-neoplastic conditions are frequently met with in different pathologies, so that it may become possible to discount a positive result, obtained with test A, when in the same case test B reacts negatively. Some of the methods described have been reported so as to permit distinction between the two most important groups of malignant tumors, viz., between carcinomas and sarcomas. Also claims, which go farther, have been advanced—namely as to a possibility of ascertaining the exact localization of the diagnosed neoplasm by means of tests, but convincing proof for such an accomplishment is as yet lacking and may hardly even be expected to lie within the range of possibility. On the other hand, serious consideration must be given to the fact that the general term of neoplasia in reality comprises a great variety of pathologies which, though doubtless characterized by common fundamental criteria, are distinguished from one another by their gross anatomy and by their histological type, and which undergo a series of successive stages exhibiting different aspects and affecting the body of the tumor host in a different manner. Hence, it appears highly problematic whether a single test will be found capable of uniformly determining the presence of all these various forms of malignant tumors, so that this circumstance may again be advanced as a reason which makes advisable the combined performance of several diagnostic tests in every single case.

Two factors, as the authors view them, appear to be necessary postulates which should be fulfilled for the purpose of securing the desired efficiency of combined tumor diagnostics. One of these conditions may be defined as the requirement that each of the partial tests chosen should be derived from a different theoretical basis, involving different phenomena and mechanisms connected with the tumor process. For, only in this manner, may it be feasible to eliminate sources of error due to unspecific reactions in control cases or to negative findings in tumor patients—errors which appear because of shortcomings of one of the tests employed. Secondly, it is deemed extremely desirable that diagnostic studies of this kind should be carried out in special laboratories or institutes which are not only equipped and staffed in the best manner available, but which work in close cooperation with clinics and hospitals. Many failures of the past may have been partly due to a neglect of these factors, be it that the investigations were performed in makeshift laboratories by clinicians who could devote only part of their time to this complex work, or because efforts of this kind have been undertaken by research workers engaged mainly in theoretical problems who lacked the cooperation of hospitals necessary for a thorough insight into the clinical and biological aspects of the cases tested.

Although it is comprehensible that the methods discussed in this chapter have been primarily employed for detecting neoplasia at an early time, or for deciding whether a certain pathology possesses malignant character, it should not be forgotten that these procedures, if proven reliable to a certain extent, would represent a valuable asset in the practical management of cancer patients when put to a somewhat different use. This refers to the possibility of employing the tests for purposes of prognosis, that is, of gaining insight into the advancement of the neoplastic process by ascertaining the quantitative deviations from the normal as indicated by the outcome of tests performed repeatedly during the course of the disease. This approach has been tried by some investigators, but it could certainly be utilized to a much larger extent and might furnish valuable information, particularly as to the formation of metastases, or the development of recurrences in patients following surgical treatment. Generally, such methods might also be useful for the evaluation of therapeutical measures. In this respect, a more solid theoretical basis could be established, both for therapeutical and for diagnostic procedures, by testing them in adequate animal experiments which can be more accurately controlled than human pathology. On the one hand, diagnostic tests would prove their reliability if it were possible to demonstrate that therapeutical measures of known efficiency would be followed by a return to the normal of the test under investigation. On the other hand, similar experiments could be employed for studying the effectiveness of a less-well known or a new treatment of neoplasia. In this manner, mutual furtherance of the understanding of theoretical and practical aspects of the cancer problem may result, and, at last, a satisfactory answer may be found to this biological mystery responsible for one of the most insidious of human diseases.

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